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THE SPECIFIC TREATMENT OF CARCINOMA.[1]

(Preliminary Report.)

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Since the first experiments by Jensen made in 1903, in immunizing against malignant growths, many investigations have been undertaken on the same subject, in all of which the object of study has been a tumor of some lower animal.

Immunity of greater or less degree has been produced in the mouse, the rat, the dog and the rabbit against many different kinds of tumor; in some instances, however, the attempts to immunize have failed, or have even resulted in an increased rapidity of growth.

It has been found that while the most marked degree of resistance to the development of transplanted tumor cells is called forth by previous inoculation with the protoplasmic substances of the particular tumor under investigation, a good measure of resistance can be produced by the injection of normal tissues, adult or embryonal, provided they be of the same class as the tumor, i. e., epithelial or connective tissue as the case may be. Studies by Ehrlich seemed to show that the induced immunity to tumors in lower animals is not absolutely blasto-specific; but this view has been controverted by Bashford who showed that only the epithelial covering of the embryo, not the rest of the body, could be successfully used in immunizing against epithelial tumors.

[1] To be read before the annual meeting of the Manila Medical Society, January 3, 1910.

Among the more recent investigations upon immunity to tumors, those of von Dungern [2] are perhaps the most significant in their bearing upon the problem of immunization against cancer in human beings.

Von Dungern excised a young, rapidly growing, moderately malignant sarcoma, which had been derived originally from a wild hare, and had been transplanted to the laboratory rabbit, and inoculated with it two groups of rabbits. Group A consisted of normal, i. e., hitherto unused animals; group B had been previously inoculated with an emulsion of an identical tumor derived from another transplant. Among the second group were animals in which the tumor resulting from the first inoculation was still present, as well as animals in which the resulting growth had been excised, or had been spontaneously absorbed, and also animals in which the first inoculation had failed altogether. Although every individual of group A developed large tumors, in not one animal of group B was the second inoculation successful.

Furthermore, the tumors still present in some of the members of group B, which were derived from the primary inoculation, softened after the second injection of tumor material and were converted into sterile abscesses.

The predominant cell found in these softened tumors was the macrophage, which was accompanied by a few lymphoid and plasma cells.

Von Dungern believed that the immunity against the second inoculation was due to the development in the previously inoculated animal of a condition of hypersensitiveness, as a result of which the second introduction of tumor material was met with an exaggerated local inflammatory reaction. He thought he could demonstrate, at the site of the second injection, the presence of nonspecific cytolytic substances which he considered to be produced locally as a part of the exaggerated reaction.

The chief object of all studies upon immunity against cancer is, naturally, the finding of some means by which this fearful disease in human beings may be successfully combated.

While all these experimental results are suggestive in their bearing upon the cancer problem in this restricted sense, none of them correspond strictly with the conditions existing in human beings afflicted with this disease. In man we are dealing with primary growths, whereas in lower animals the experiments have all been made with transplanted tumors.

The all-important problem of cancer research has yet to be solved, namely, is it possible to immunize human beings against their own primary malignant growths? Experiments that throw light upon this question have already been published.

The earliest of these were those of Metalnikoff, who in 1900 demonstrated the production in guinea pigs that had been injected with guinea pig's testicle, of specific antibodies which were capable of destroying the spermatozoa of the same animal. Following these studies, von Dungern and Hirschfeld,[3] after having

[2] *Ztschr. f. Immunitatsf. Orig.* (1909), 2, 391.

[3] *Centralbl. f. Bakt. etc. Ref. Beilage zu Abt. I.* Jena (1909), 44, 57.

excised one testicle of a rabbit and having injected an extract of the organ into the same animal, were able to demonstrate the development, in the treated rabbit, of a condition of hypersensitiveness. According to some experiments of Gorowitz, reported by von Dungern, a condition of hypersensitiveness to the cancer proteids can be demonstrated in the patient bearing the tumor. This condition shows itself in an increased reaction, as compared with that produced by injection into normal individuals, upon subcutaneous inoculation with extracts from the patient's own tumor. No increased reaction could be elicited in these cases by the injection of extracts from the tumors of others.

At first thought it would seem improbable that an organism could be induced to elaborate substances capable of destroying its own tissues. The publications of Metalnikoff, von Dungern and Hirschfeld, and Gorowitz, however, demonstrate just this power. It can still be objected that in the experiments reported by these observers, the injurious effect of the antibodies was exerted not upon the cells of the organs *in situ,* but only when these had been isolated *in vitro.* In reply to this objection, attention may be called to the fact that the normal epithelial cells are separated from the other tissues of the body by a dense basement membrane, the function of which can be to prevent, on the one hand, the entrance into the circulation of the specific epithelial cell products, and, on the other hand, the access to the epithelial cells of any specifically inimical substances carried by the blood. The operation of the latter assumed function would explain the immunity to the destructive action of the complex cytotoxins present in the blood, which is enjoyed by the spermatozoa *in situ,* in the case of the rabbit treated with its own testicular tissue.

Entirely analogous to such a physiological condition is the fact that complement is normally not present in the aqueous humor of the eye.

One of the earliest evidences of beginning malignant change in epithelium is the disappearance of the basement membrane at the point of invasion of the underlying tissue. In the further course of the disease, this peculiarity is maintained, and the tumor cells are thus brought into closer relationship with the blood than were the normal parent cells.

The assumption of a protective function exercised by the basement membranes, taken together with the fact that in all malignant tumors this protective membrane is wanting, would sufficiently explain why the tumor cells in the immunized individual may be destroyed by specific cytotoxins in the blood, whereas the normally situated epithelium is spared.

The experiments of von Dungern upon the sarcoma of the wild hare would seem to stand in direct contradiction to the hypothesis here advanced. It is to be borne in mind, however, that the immunity demonstrated by him against the sarcoma was always against inoculation of tumor tissue taken from another animal, and had in every

instance been produced by injections of sarcoma material derived from a similarly foreign source. In most cases the biological relationship between the tissues of the animal to be immunized and that of the sarcoma was a comparatively distant one, and even in the few experiments in which the immunity seemed to be demonstrable in an animal of the same species as the one in which the tumor originated, the material used both for immunizing and for testing the immunity had been growing for some time in a biologically foreign organism.

However, if it is found to be possible to immunize an individual against his own sarcoma, then, of course, the hypothesis here suggested will have to be modified.

Another explanation of this apparently paradoxical phenomenon has been suggested by von Dungern,[4] namely, that the tumor cells may be so altered in biological character from the normal parent cell that the antibodies elaborated against the former may be specifically unrelated to the latter. The superiority of malignant tissue over normal tissue in its power of inducing immunity against tumors would speak in favor of this view.

Theoretical speculations as to the probable outcome of an experiment, while often of value in determining the manner of procedure, are usually less satisfactory than the experiment itself.

In order to immunize a cancer patient against his own cancer, it is necessary, according to our present knowledge of immunity, to inject into his body some of the tumor tissue or an extract from the tissue.

Similar attempts to induce, in human beings, by inoculation an active immunization against their own tumors have already been made. The first of these were reported by von Leyden and F. Blumenthal.[5]

An exact description of the technique of their treatment does not appear in their publication. In none of their cases did they produce a complete cure of the disease, although they believed that in three instances, as a result of the treatment, metastases were prevented, and affected lymph glands reduced in size. These experiments were apparently discontinued.

Von Dungern[6] in his paper, "Untersuchungen uber das Wesen der Immunität gegen Karzinom," read June 4, 1909, in Vienna, stated that such experiments had been instituted in the cancer hospital at Heidelberg, and Ranzi, in the subsequent discussion, referred to his own treatment of a few tumor patients with carbolized extracts of their own tumor tissue. So far as we are aware, neither of these two investigators have made any report as to the results of their treatment.

In our earliest experiments we were apprehensive as to whether we might not reinfect the patient with cancer at the point of injection, and also that large quantities of cancer extract might be directly toxic if

[4] Oral communication.

[5] *Deutsche med. Wchnsch.* (1902), **28**, 36.

[6] *Centralbl. f. Bakt.* etc. *Ref. Beilage zu Abt. I.* Jena (1909), **44**, 57.

absorbed all at once into the circulation. Furthermore, we feared the possibility of infecting the patients with bacteria with which the material to be used for inoculation might have become contaminated, either before operation, as in the case of any surface growth, or during the preparation of the tissue for injection.

All of these fears have proved groundless. No tumors have appeared at the point of the injection of the cancer material, and in view of the active immunity to the development of the tumor which is called forth by the inoculation of such material, such a contingency need hardly be further considered. Although large quantities, 10 to 15 grams of cancer tissue have been injected, no toxic effect has been observed. Thus far bacterial infection has not occurred. As an antiseptic measure we have found the addition of 1 cubic centimeter of 5 per cent carbolic acid to every 10 cubic centimeters of fluid entirely adequate. The only departure from the rule in this regard was at the first operation, August 14, in Case 7 of group IV. In this instance the tumor material was vigorously shaken with an excess of toluene and left in contact with this disinfectant for about six hours, after which time the toluene was driven off at a temperature of 38°C. By this procedure apparently all the proteids were precipitated. In spite of a large injection of this preparation, recurrence of the tumor followed within four months.

The "vaccine" which we have used in our experiments has been prepared as follows:

> The tumor tissue, immediately after excision, is comminuted as far as possible with scissors and passed ten times through a vaccine grinder which has been sterilized by successive rinsing with pure carbolic acid, absolute alcohol, and sterile salt solution. After the first passage through the grinder an equal volume of sterile salt solution is added. By this thorough grinding of the tissue practically all the tumor cells are completely broken up so that the decanted fluid contains all the protoplasmic and nuclear substances of these cells. In order to get rid of the shreds of fibrous tissue the thick, creamy fluid is centrifugated at a high speed for three minutes and decanted.

The injection is made preferably within six hours after the addition of the carbolic acid. If it is reasonably certain that contamination of the vaccine has not occurred, the injection may be made at once without the addition of carbolic acid.

The largest amount of tumor tissue injected at one time was from about 20 to 25 grams. In this instance the tumor contained practically no fibrous tissue, so that almost the entire quantity of the material taken for the "vaccine" could be used for treatment. When such large amounts are injected, they can be distributed conveniently in several (four to six) places in the subcutaneous tissue of the abdominal wall. In some instances we have divided the material into two portions, reserving one for a second treatment three weeks after the first. Whether the material, after three weeks standing in contact with the carbolic acid,

still retains its specific biological characters, we do not know. However, the efficacy of a single large injection is amply shown in cases 2, 3, and 4 of group IV.

The degree of local reaction of the tissues to the injections has varied from absolutely none whatever (case of Hodgkin's disease) to abscess formation (two cases of carcinoma). In neither of these latter cases were the ordinary pus organisms found. One was sterile (five cultures having been taken on agar and blood serum) and the other, situated in the subcutaneous tissues of the abdomen and opened four hours after death, contained only *B. coli communis* and *B. proteus vulgaris.*

In one case, following the experiments of Gorowitz, a normal individual received at the same time as the cancer patient the same injection. The local reaction in the control was, if anything, more vigorous than in the patient from whom the cancer tissue was derived. This single experiment, however, can not be considered as contradicting the investigation of Gorowitz, since, in our case, the amount injected was so great as possibly to conceal the small differences that might be evident after the injection of smaller quantities.

We have divided the fourteen cases which form the basis of this preliminary report into four groups in the hope of conveying a clear understanding as to the type of case with which we were dealing in each instance, as to the extent of the effect which the "vaccine" has exerted, and, finally, as to what would have been the probable course of the disease if surgery unaided had been depended upon. In group I we have included those cases which were considered curable by operation, where the growth and its visible extensions were readily and naturally removable. In group II we have placed those cases probably curable by operation, those in which the removal though possible was more difficult by reason of the infiltrating character of the growth. In group III we have placed those of doubtful curability by operation, those cases where even with radical measures carcinomatous material was probably unavoidably overlooked and not removed. In group IV we have included those positively inoperable tumors, in which the extent of the growth absolutely precluded its complete removal and definite carcinomatous material was seen to remain in the wound.

GROUP I.—CASES CURABLE BY OPERATION.

CASE 1.—No. 7177. Filipino aged 80 years. Ulcerated carcinoma of right forearm with metastases to glands at elbow. Operation May 19, 1909, complete removal of all visible carcinomatous tissue and glands. *Inoculation of cancer vaccine May 19, 1909.* No signs of recurrence on January 3, 1910.

CASE 2.—No. 7419. Filipina aged 39 years. Carcinomatous degeneration of ulcerated fibro-myoma of cervix, with no visible involvement of lymphatics. Operation, pan-hysterectomy, July 5, 1909. *Inoculation of cancer vaccine July 5. 1909.* No signs of recurrence January 3, 1910.

GROUP II.—CASES PROBABLY CURABLE BY OPERATION.

CASE 1.—No. 8505. Filipina aged 50 years. Ulcerated buccal carcinoma with metastases to cervical lymph glands. Operation December 1, 1909; complete removal of all carcinomatous material and glands of neck and closed by plastic flap. *Inoculation of cancer vaccine December 1, 1909.* No indications of recurrence have appeared.

CASE 2.—No. 8162. Filipina aged 45 years. Medullary carcinoma of right breast with involvement of skin and axillary glands. Operation by Dr. Schiffbauer October 13, 1909. Jackson operation for complete removal of breast and axillary glands. *Inoculation of cancer vaccine October 13, 1909.* Patient was heard from December 28, 1909, at which time there were no signs of recurrence.

GROUP III.—CASES OF DOUBTFUL CURABILITY BY OPERATION.

CASE 1.—No. 8197. Filipina aged 44 years. Adeno-carcinoma of left breast with involvement of pectoral muscle, skin, and axillary glands. Operation by Dr. Schiffbauer October 20, 1909. Jackson operation for removal of breast, pectoralis major and minor, and axillary glands. *Inoculation with cancer vaccine October 20, 1909.* Patient was still well on January 3, 1910.

CASE 2.—Filipino aged 67 years. Adeno carcinoma of right breast involving, by metastasis, fully one-third of pectoralis major muscle, and the axillary and retroclavicular lymph glands. Operation December 6, 1909; excision of breast, outer half of pectoralis major muscle and majority of axillary glands. *Inoculation with cancer vaccine December 6, 1909.* No signs of recurrence January 3, 1910.

GROUP IV.—POSITIVELY INOPERABLE CASES.

While it is too early to draw any conclusions from any of the above cases the results we have obtained in the cases presented in this group are already of significance.

CASE 1.—No. 7052. Filipino aged 51 years. Recurrent carcinoma of right cheek. This patient had been operated upon in the clinic at St. Paul's Hospital and the growth had promptly recurred within three weeks' time, while the patient was still an inmate of the ward. At the second operation only a portion of the growth was removed to use in making a vaccine, as so much tissue had been sacrificed at the previous one, that a plastic operation the second time was impossible. No vaccine treatment had been given at the time of the first operation. *Second operation and inoculation with vaccine were made June 14, 1909.* On December 29, 1909 there were no signs of further growth or recurrence in the cheek, complete healing of carcinomatous areas having taken place, with disappearance of thickening.

CASE 2.—No. 8175. Filipino aged 45 years. Carcinomatous cyst of left neck involving, by metastasis, all deep structures including deep vessels, also the overlying skin to a marked degree. Operation November 6, 1909; tumor removed as completely as possible by block dissection of neck; carcinomatous material remained at upper angle of wound, adherent to upper segments of trachea, and to greater corner of hyoid bone. *Inoculation with cancer vaccine November 6, 1909.* Three days after the operation a nodule was noticed at the upper angle of the incision, the nodule measuring 2 centimeters in length and lying about 3 centimeters from the line of incision. The nodule was immovable, hard and lay evidently below the skin. It increased in size until, on the seventh day after the operation, it was distinctly larger and less movable. One week later it had

become smaller, softer and more freely movable. At the end of another week—i. e., two weeks after the injection of vaccine—a section extending to the center of the tumor was removed for histologic examination. This showed a dense, thick, fibrous capsule and central reticulum, in the meshes of which were found nests of large cells in all stages of degeneration and necrosis together with invading lymphoid and plasma cells, and macrophages. January 3, 1910; the nodule in the neck has not increased in size, is freely movable, and feels as if it were a mass of scar tissue. Healing is otherwise complete and there are no signs of recurrence.

CASE 3.—No. 8444. Filipino aged 44 years. Carcinomatous cyst of left neck involving structures practically similar to case No. 8175. *Operation and inoculation of vaccine November 23, 1909.* January 3, 1910, neck is completely healed and soft and there are no signs of carcinoma, although at the operation a definite portion of tumor tissue was left adherent to the sheath of the vessels and to the horn of hyoid bone.

CASE 4.—No. 8326. Filipina aged 71 years. Advanced carcinoma of left buccal mucosa involving entire cheek and, by extension, the side of the neck. Operation November 10, 1909; removal of as much tumor as possible and left half of lower jaw; block dissection of the neck. Carcinomatous tissue remained unexcised at the base of the tongue and left pillar of fauces. *Inoculation of vaccine November 10, 1909;* death from intercurrent affection December 18, 1909. At autopsy absolutely no sign of malignant tissue was found locally or anywhere in the body, the unexcised portions of malignant tissue having disappeared from the base of the tongue and from the pillar of fauces.

CASE 5.—No. 8442. Spaniard, male, aged 40 years. Carcinoma springing from some deep epithelial structure of right upper neck, involving all deeper structures, parotid and submaxillary glands, and overlying skin. At the operation December 7, 1909, it was found impossible to remove all of the malignant tissue. In addition to a mass surrounding the great vessels, two large nodules, measuring about 2.5 by 3 centimeters were left superficially, one posterior to and below the angle of the jaw and the other in the region of the submaxillary gland extending outward to the level of the cheek and upward behind the jaw toward the base of the tongue.

Injection of vaccine was made December 7, 1909. On the seventh day thereafter the first nodule was found to be converted into a soft, fluctuating area suggesting a pocket of pus, although lacking all signs of inflammation. The wound had healed cleanly by first intention. This fluctuating area opened spontaneously through a small passage left by a stitch opening. Cultures taken from the contents the next day showed *Staphylococcus aureus* and *albus*. Under simple dressings the area has healed and the nodule has entirely disappeared. The second nodule in the submaxillary region has grown gradually smaller and less board-like until to-day, January 3, 1910, it has almost disappeared and the skin and superficial structures, excepting in one small area, have become normally soft. The patient is able to swallow with much greater ease than he could before the treatment. He is still in the hospital under observation.

CASE 6.—No. 8233. Filipino aged 49 years. Fungating carcinoma involving practically the whole of the right neck in its lower half and extending behind clavicle. First operation October 30, 1909, at which time apparently the entire growth was removed. No treatment by vaccine was given as the material was spoiled. There was a prompt local recurrence within a week.

The second operation was performed when sufficient amount of tumor had grown to supply material for making the vaccine. Only the most superficial

portions of the fungus were scraped away as the patient was in very bad condition. At this time a tumor of the right shoulder was observed, which had previously escaped notice. This mass extended over the entire shoulder so that the contour was not very markedly altered. This shoulder mass grew rapidly within the next five days and was hard and brawny; the overlying skin became infiltrated and roughened and the arm œdematous.

On the seventh day after treatment with the vaccine there was noted a marked local disturbance in the shoulder. The skin temperature of the carcinomatous area was markedly increased above that of the rest of the body, which was normal. The tumor was painful, the skin darker and congested, and the entire mass distinctly softer.

The following day, the eighth after treatment, the temperature difference was less marked, the pain less, and the softening more pronounced, amounting almost to fluctuation. Under cocaine anæsthesia the shoulder was incised for the purpose of obtaining material for microscopic and bacteriologic examination. Cultures remained sterile; smears showed the presence of only a small number of polymorphonuclear leucocytes, and large numbers of epithelial-like cells. Sections of the excised tissue showed typical carcinomatous growth identical with that of the original tumor. Several recurrent masses along the upper portion of the neck wound had, during this period, undergone softening and atrophy. The main wound remained clean and free from recurrence, the edges had advanced a centimeter and a half toward the center by a growth of apparently healthy epithelium and a large carcinomatous mass left behind the clavicle became markedly discolored and shrank perceptibly in size. The patient died four days later. Autopsy showed a tumor mass in the mediastinum that had not been noted, and the cause of death to have been hydropericardium, right hydrothorax, and œdema of the left lung.

CASES 7 AND 8.—Nos. 8405 and 8497. These cases are under treatment at present. In each of them, large masses of carcinoma, involving the bladder and ureters, the rectum, and pelvic fasciae, were present and only portions were removed for making a vaccine.

Too short a time has elapsed to make it possible to anticipate the effect of the treatment upon the course of the disease. In cases 7 and 8, however, this much can be said: in each of the cases the marked cachexia that was present before the treatment has entirely disappeared.

The disappearance of the cachexia, which was noted within three days after treatment, must be looked upon as a remarkable phenomenon. According to all theories hitherto advanced in explanation of tumor cachexia, the injection of large quantities of tumor material should have increased rather than diminished the symptoms.

The experiments of Gorowitz, together with those of Friedberger dealing with the nature of anaphylaxis, seem to offer the most satisfactory basis of explanation for the cachexia of malignant tumor. Gorowitz has demonstrated in cancer patients a condition of hypersensitiveness to the cellular substances of their own tumors. This condition was absent in normal individuals. We are forced thus to conclude that sufficient quantities of the tumor cell substances enter the circulation to produce in the body the condition referred to.

Friedberger,[7] on the basis of strong experimental evidence, believes that the injurious effects of the second injection, in the condition of anaphylaxis, are due to the entrance into the sensitized cells of the previously treated animal's body, of its own complement. This entrance of complement follows the union of the antigen, introduced at the second injection, with the specific antibodies which are present in the sensitized cells.

The mechanism, therefore, of production of the tumor cachexia, according to the experimental data at our disposal, would be, first, the establishment of a condition of hypersensitiveness to the epithelial substances of carcinomata due to the absorption of these materials in small quantities; secondly, the continual entrance of complement into the sensitized cells as a result of the constant absorption of tumor material.

According to this conception, the disappearance of the cachexia following the injection of large quantities of tumor substance is to be looked upon as corresponding with the condition known as the state of antianaphylaxis.

The introduction of the large doses of tumor material results in the formation of an excess of antibodies which are then thrown out into the circulation. The absorption of these antibodies by the tumor cells transfers the scene of injurious complement activity from the normal cells to the malignant ones.

As already implied, the assumption of von Dungern that the immunity against malignant growths is due to a local reaction of hypersensitiveness, does not seem satisfactorily to explain the results obtained by us in the foregoing experiments. These, we believe, may be best explained by assuming the formation of antibodies in excess, their transportation to the malignant cells by the serum and the resulting destructive action upon these cells of the patient's own "complement."

If this is true, the rational treatment of those cases in which large tumor masses have to be left in the body would be the successive injection at intervals of two weeks (at the end of which time the available antibodies will have been used up) of large quantities (5 to 10 grams) of tumor material. The ideal method of preserving the tissues for this purpose is by freezing.

In only one of the cases of carcinoma in the region of the neck in group IV was the carotid artery ligated, case No. 8326. At autopsy thirty-nine days after operation the carotid in this case was found to be patent.

Only one case of malignant growth other than carcinoma has come to treatment, a very advanced case of Hodgkin's disease. This case was apparently uninfluenced by treatment.

[7] *Ztschr. f. Immunitätsf. Orig.* (1909) I, 2, 208 *Ibid* (1909), 3, 581, 692.

Our thanks are due Dr. John R. McDill, Professor of Surgery, Philippine Medical School, who has placed at our disposal all cases of malignant disease admitted to his clinic since we began this work.

SUMMARY AND CONCLUSION.

1. The protoplasmic substances of malignant epithelial tumors in human beings can be injected subcutaneously in large quantities without injurious results.

2. In three cases affected with carcinoma, such injections have been followed by the softening and disappearance of tumor masses measuring in diameter from 2 to 4 centimeters.

In a fourth case, a rapidly growing lump appeared near the site of the excised cancer two days after treatment. Several days later, when the lump had reached a diameter of 4 centimeters, the growth ceased and subsequent microscopic examination showed it to be a firmly encapsulated mass of dying epithelial-like cells.

Three other surgically absolutely inoperable cases, in two of which visible amounts of cancer tissue were left unexcised, have remained until now, i. e., for from five weeks to six months, free from recurrence of the disease.

Seven other cases have remained free from a return of the disease for from one to seven months. Two of these were designated as "curable by operation;" three were designated as "probably curable by operation;" and two were designated as "of doubtful curability by operation."

In only one case has the disease returned after treatment. In this case the material used for vaccination was rendered inert by too vigorous disinfection.

Hitherto no case of sarcoma has come to treatment; however, in view of the failure of the procedure to affect the course of Hodgkin's disease, the outlook for sarcoma is not promising.

3. Tumor cachexia is not increased by the rapid absorption of large quantities of tumor tissue; on the contrary, large injections of tumor material seem to cause the disappearance of the cachexia.

While this paper was in press further experiments seem to indicate;

(1) That the efficacy of the treatment is *indirectly* proportional to the amount of malignant tissue left in the body, and, within certain limits, *directly* proportional to the amount of tumor-cell substances injected and to the number of such injections made.

(2) That a carcinoma in one individual can be successfully treated with injections of material from a carcinoma of the same kind taken from *another* individual.

(3) That one-half per cent of carbolic acid does not destroy the "vaccine" within thirty-six hours if the latter is kept at a temperature of 7° centigrade.

NOTE.—No attempt has been made in this preliminary article to consider the results of all of the experimental work performed on animals with transplanted tumors, notably among which may be mentioned those obtained by Gay, *Journ. Med. Research* (1909), **20**, 175, *Boston Med. Surg. Journ.* (1909), **161**, 207. All of the work will be referred to in the more complete paper to be published later in this Journal.

CO-EDITOR.

THE CULTIVATION OF THE LEPROSY BACILLUS.

By Moses T. Clegg.

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At the Sixth Annual Meeting of the Philippine Islands Medical Association which was held at Manila, February 11, 1909, I made a preliminary report [1] upon the cultivation of the *Bacillus leprœ*. In this report I stated that I had succeeded in cultivating, from the spleen and skin nodules of typical cases of leprosy, an acid-fast bacillus which grew upon agar in symbiosis with amœbæ. The amœbæ had been obtained from a dysenteric stool and were cultivated with the cholera vibrio.

At the time of the first publication, the acid-fast organism had been obtained in symbiosis with amœbæ from the spleen in two cases and from skin nodules in three other cases of leprosy, but had not been isolated in pure culture. Since then similar cultures have been obtained from leprous tissue in three additional cases and from two of these the acid-fast organism has finally been isolated in pure culture.

In continuing the work of cultivation I have used the same technique described in the preliminary report, always making the original inoculations from the leprous tissues upon cultures of amœbæ with the cholera vibrio.

Among the sources of experimental error which were mentioned in the previous communication was the possibility that the acid-fast organism had been growing originally with the amœbæ in the human colon. In order to rule out this possible source of error amœbæ obtained from a different source than those used in the first experiments, namely, from the tap water, have been cultivated with the cholera vibrio and these cultures used for the original inoculations with leprous material.

No acid-fast bacteria have been found in the cultures of the water amœbæ excepting after inoculation with material from a case of leprosy, so that the source of error under discussion can be excluded. Furthermore, since the cultivation of the acid-fast organisms has been successful with amœbæ obtained from a case of dysentery as well as those from tap water, it is not probable that any importance attaches to the strain of amœba used. It is important, however, for the successful cultivation

[1] *This Journal, Sec. B* (1909), 4, No. 2, 77.

of the acid-fast organism to have a good growth of amœbæ for the original inoculations.

In the cultivation of amœbæ the following medium, as described by Musgrave and Clegg,[2] was used:

Agar	20.
Sodium chloride	3.0
Extract of beef	3.0
Water	1,000.

This is prepared in the same manner as ordinary nutrient agar, the finished product being made 1 per cent alkaline to phenolphthaleïn.

The medium is placed in tubes and sterilized. Before inoculating with the material containing the amœbæ the medium is melted and poured into sterile Petri dishes and allowed to harden. The material containing the amœbæ is then streaked over the surface of the plate. In cultivating amœbæ from water and other external sources, 100 to 500 cubic centimeters of water, or the same amount of an aqueous solution or suspension of other substances is collected in sterile flasks to which is added 1 cubic centimeter of alkaline bouillon for every 100 cubic centimeters of the sample. The flask is then set aside for from twenty-four to forty-eight hours, when an examination will usually show the presence of amœbæ on the surface of the liquid. A loop of this culture is then streaked over the plates already referred to. The plates are then kept at a temperature not to exceed 37° C. The temperature used for incubation must be regulated according to the growth of the symbiotic bacteria; if the latter are found to grow so profusely at a given degree as to interfere with the development of the amœbæ, then a lower temperature must be used. After incubating twenty-four to forty-eight hours the amœbæ may be detected by examining the plates under the low power of the microscope. After two or three days' growth of the amœbæ upon agar, transplants are made to fresh plates of the same medium. When a suitable growth of the amœbæ and a mixed culture of bacteria has been procured, the next step necessary is the isolation of the amœba with one kind of bacteria. This may be accomplished in the following manner: The sterile medium for cultivating amœbæ is melted and poured into sterile Petri dishes and allowed to harden. With a platinum loop several rings of a pure culture of the organisms with which it is desired to grow the amœba are made on the surface of the hardened agar, and a small smear inoculation of the mixed culture of the amœba is placed on the middle of the smaller or central bacterial ring. If the usual precautions have been taken, most amœbæ, as they multiply, will generally spread rapidly over the plate and, in passing through the bacterial rings, will lose to a certain extent the bacteria with

[2] *Pub. P. I. Bur. Sci. Biolog. Lab.* (1904), **No. 18**, 9–78.

which they started and take up those forming the rings. In from twenty-four to forty-eight hours the amœbæ will have passed to the outer ring and from this location they may be taken for transplantation to fresh ringed plates with a medium suitable for their growth, the same procedure being repeated until by plating it is shown that the amœba is growing only with the bacterium desired. In continuing the work on the cultivation of the leprosy bacillus, as has been mentioned, an amœba was used which had been isolated from the city water supply and was growing in symbiosis with the cholera vibrio.

The mixed culture of the amœba and the cholera vibrio was inoculated on slant tubes of plain sterile agar-agar without the beef extract, but containing the same amount of sodium chloride and having the same reaction as the amœba medium previously described—and after twenty-four hours' growth of the mixed culture on this medium, material containing leprosy bacilli was added; at the same time control inoculations of the leprous material were made upon the same medium without the amœba and the cholera vibrio. The tubes were incubated for one week at 37°, at the end of which period examination showed an increase in numbers and change in morphology of the acid-fast bacilli in three of the amœba cultures, whereas on the plain agar these bacilli had changed neither in number nor in morphology.

After having been transplanted once a week upon amœba-cholera cultures for six weeks in one case and three months in another, the acid-fast organisms were obtained in pure culture by heating; in this manner the cholera vibrios and amœbæ were killed by a temperature of 60° C. for half an hour, while the acid-fast bacilli survived and grew readily alone, when transplanted.

The acid-fast organism was obtained from the two following cases:

Case F.—A well-marked case of tubercular leprosy. The autopsy was performed three hours after death. The lungs showed the presence of tubercular cavities and extensive adhesions to the chest wall. No lesions of tuberculosis were found in the other organs. The spleen was removed under aseptic conditions by the following method: A flap of the skin on the left side covering an area extending from the median line to the vertebral column and from the axilla to the crest of the ilium was removed. Alcohol was then poured over the muscular layer and burned, after which the peritoneal cavity was opened with sterilized instruments and the spleen removed and placed in a sterile vessel. Cultures were then made in the usual way, after first burning the capsule of the spleen and puncturing it with a sterile knife.

Ten tubes, each containing a 24-hour growth of amœbæ and the cholera vibrio, were inoculated with the splenic pulp as were also ten control tubes containing sterile agar. All the tubes were placed in the incubator and kept at a temperature of 37° C. for seven days. At the end of this period smears from the tubes containing the mixed culture of amœba and cholera vibrio showed, when stained by the Ziehl-Neelson

method, a change in the morphology and a multiplication of the leprosy bacilli, while smears made from the control tubes stained in a similar manner showed no evidence of a multiplication of the bacilli inoculated and no noticeable changes in morphology. Transplants of the mixed cultures of amœba, cholera and leprosy bacilli were made to fresh agar tubes and incubated at the same temperature. Smears from these tubes made three days later and stained as before showed a great increase in number of the acid-fast bacilli and also a change in their morphology from the long, slender bacilli found in smears from leprous lesions to short plump rods and occasionally coccus forms. This deviation in morphology is not constant, however, on continued cultivation; some cultures have resumed a more typical morphology, as is shown in the illustrations. (Plate I, fig. 1.) Transplants of the mixed cultures were then made once a week for three months. After having been on the artificial medium for this length of time, the cultures were placed at a temperature of 60° C. for thirty minutes. This temperature was sufficient to kill the amœbæ and the cholera vibrios, but not the acid-fast bacilli. Transplants were made from the heated cultures to fresh tubes containing amœbæ and cholera vibrios and also to tubes containing plain, sterile agar. After three days' incubation, smears from the tubes containing the amœbæ and cholera vibrios showed a development of the acid-fast bacilli, and at the end of six days small, brownish colonies with regular margins appeared on the surface of the tubes containing plain agar only. Smear preparations made from these colonies and stained by the Ziehl-Neelson method showed microscopically an acid-fast bacillus, the cultural characteristics of which will be described in another part of this paper.

Case G.—This was a well-marked case of tubercular leprosy. The autopsy was performed two hours after death. A small nodule was present in the apex of the left lung which might have been of tubercular origin; there were no evidences of gross tubercular lesions in the other organs. The spleen was removed in the same manner as described in Case F. Smear preparations from the spleen stained by the Ziehl-Neelson method showed very few leprosy bacilli. Cultures were made on media containing amœbæ and cholera vibrios and on plain sterile agar. Because of the small number of leprosy bacilli present in the spleen, a large nodule situated in the thigh and containing large numbers of the organism was also selected for use in making the inoculations and was excised. In making the cultures the surface of the nodule was first burned with a hot spatula and an opening made with a sterilized scalpel.

Ten tubes containing amœbæ and cholera vibrios were inoculated with material taken from the center of the nodule, as were also ten tubes containing plain agar. All these cultures, together with several tubes containing amœbæ and cholera vibrios, but not inoculated with leprosy material, were placed in the incubator at a temperature of 37° C. At the end of six days smear preparations from two of the tubes containing amœbæ, cholera vibrios and material from the nodule showed a change in morphology and an evident multiplication of the leprosy bacilli.

All other tubes, including those made from the spleen, were unchanged and remained so. Transplants were made from the two tubes showing a growth of the leprosy bacillus with the amœba and the cholera vibrio, to fresh agar tubes and kept at the same temperature. Smear preparations made at the end of the third day showed a great increase in the number of the acid-fast bacilli and the same change of morphology as was observed in the previous case. Negative results were obtained in transplants from both sets of control tubes, namely, from those containing amœbæ and cholera vibrios, but no leprosy material, and from those containing plain agar inoculated with material from the nodule. The tubes which contained amœbæ and cholera vibrios and which were inoculated with the splenic material were also negative. Weekly transplants were then made from the two tubes showing a growth of the acid-fast bacillus with the mixed culture of amœba and cholera. After having been growing in this mixed symbiosis for six weeks, these cultures were subjected to a temperature of 60° C. for thirty minutes, and as in the previous case, the acid-fast organisms were obtained in pure culture.

Case H.—A mixed type of leprosy. The autopsy was performed three hours three hours after death. The lungs showed tubercular lesions, but there was no evidence of tuberculosis in the other organs.

The spleen was removed by the method described in Case F and cultures were made in a similar manner. Smear preparations made from this spleen showed a small number of leprosy bacilli present. After three weeks' incubation smear preparations from the cultures showed no change in the morphology of the leprosy bacilli inoculated and no evidence of growth.

Case I.—A well-advanced case of tubercular leprosy. The autopsy was performed twenty-four hours after death. The lungs showed the presence of tubercular lesions; there was no evidence of other tuberculous lesions in the body. The spleen was removed aseptically and cultures were made on media containing amœbæ and cholera vibrios and also on media composed of plain sterile agar. After six days' incubation a slight growth was observed in one of the tubes containing the amœbæ and cholera vibrios. No development occurred, however, in transplants from this tube.

CULTURAL CHARACTERISTICS.

The separation of the acid-fast bacilli from the amœbæ and cholera vibrios was accomplished, as already indicated, in a very simple manner; viz, by killing the two latter organisms by subjecting the mixed cultures for half an hour to a temperature of 60° C. At this temperature the acid-fast bacteria are not killed in the first half hour.

From these heated cultures transplants were made upon amœba-cholera cultures and upon plain agar slants without the amœbæ. In both cases a growth of the acid-fast organisms took place.

Cultures were made from these original pure colonies upon ordinary nutrient agar, plain agar, potato, alkaline litmus-milk, glucose-agar,

glycerine-agar, Loeffler's blood serum, lactose-agar, Dorsett's egg medium, glucose-bouillon and glycerine-bouillon. The optimum temperature for growth was 37° C.; growth will take place at a lower temperature, but more slowly.

Surface colonies on nutrient agar.—These colonies when they first become visible are raised and possess smooth edges. They measure about 1 millimeter in diameter, are compact in appearance and, under the low power of the microscope, show a brownish pigment which is more plentiful in the center of the colony than at the periphery.

Nutrient agar stroke.—A slightly raised, moist growth appears after three days, producing a brownish-yellow pigment.

Plain agar stroke.—Growth appears after three days as slightly raised, discrete colonies, which later become confluent and moist, producing a bright orange pigment.

Potato.—After three days a raised, moist, creamy growth appears, producing a bright orange pigment. No change in the medium occurs.

Alkaline litmus-milk.—Growth occurs on the surface of the medium as a yellow membrane. The milk gradually loses its color, but does not become acid. A sediment is deposited at the bottom of the tube.

Glucose-agar stroke.—An abundant growth appears on the third day as a raised smooth moist surface with a bright orange pigment. The growth can be lifted in heaps on a platinum loop and may be readily suspended in an aqueous solution.

Glycerine-agar stroke.—Growth is similar to that on glucose agar. It may develop from a single stroke to such an extent that the entire surface of the medium is covered, giving the appearance of a heaped-up creamy substance. The growth is not adherent to the medium.

Loeffler's blood serum.—Growth is slower on this medium. It is slightly raised and moist, and very little pigment is produced. Liquefaction begins in ten days and is complete in twenty days.

Glucose stab.—Very little growth occurs along the needle tract. It is profuse on the surface of the medium.

Lactose stab.—Growth is similar to that in glucose stab. No change in the reaction of the medium occurs.

Dorsett's egg medium.—Growth is profuse on this medium after three days. The appearance is similar to that on glycerine agar.

Glucose-bouillon.—Growth appears as small patches on the surface of the medium. These later coalesce and form a wrinkled membrane. A sediment gradually forms at the bottom of the tube. The medium remains clear.

Glycerine-bouillon.—Growth is similar to that in glucose bouillon.

Fermentation tubes showed no production of gas, nor were acids formed in dextrose and lactose bouillon.

Resistance to heat.—Suspensions of the cultures in salt solution were

not killed when exposed to a temperature of 60° C. for thirty minutes. They were killed when exposed to the same temperature for sixty minutes.

The microörganism isolated from the nodule in the thigh of Case G is identical in its cultural characteristics with that isolated from the spleen in Case F.

BACILLUS FROM CASE F.

Morphology.—The length of the organisms varies from 1 to 5 microns. Stained with the method of Ziehl-Neelson, the shortest forms are colored uniformly throughout, as are also many of the long ones. In many instances, however, certain portions of the body of the bacteria are more deeply stained than the remaining ones. Sometimes, especially in the younger cultures, the center of the organism stains more deeply than do the poles, which may be only very faintly colored. Other individuals present a beaded appearance due to the alternate arrangement throughout the body of deeply and lightly stained areas. Finally, a few forms were found in which the deeply stained portions were situated in the poles.

Various shapes have been seen. Some of the very smallest approach the form of a coccus. The majority of the young bacteria are straight or slightly curved rods. Long, slim forms are also encountered. No true branching was observed and spore formation was absent.

The character of the morphology is influenced considerably by the nature of the medium upon which the organism is cultivated. The bacillus is nonmotile.

Staining reactions.—Smear preparations from cultures of all ages of the microörganism stain very faintly with the ordinary alkaline stains. Preparations made from young cultures when stained with hot carbol-fuchsin do not lose the stain when treated with Gabbet's solution. They also retain it after being washed continuously for three minutes in absolute alcohol. When treated for two minutes with a 5 per cent solution of hydrochloric acid in alcohol, a large number lose the fuchsin stain; there are others, however, which are not affected by this decolorizing agent.

Older cultures are more susceptible to the acid-alcohol decolorizing agents. Preparations from a three month's old culture stained with the carbol fuchsin solution were readily decolorized when treated with hydrochloric acid and alcohol, while smears from the same culture similarly stained and treated with Gabbet's solution or with absolute alcohol retained their color.

The bacilli in smear preparations, from lesions in guinea pigs, are acid fast when treated with hydrochloric acid-alcohol solution. Preparations from cultures of all ages were Gram positive in portions. By this

method of staining the central portions of the bacilli retain the stain to a greater degree than do the poles.

A résumé of the successful cultures including those reported in the preliminary paper follows:

Bacillus A.—An acid-fast bacillus cultivated from the spleen of a leper and growing in symbiosis with *Amœba dysenteriæ* and several other varieties of bacteria.

Bacillus B.—An acid-fast bacillus cultivated from the spleen of a leper and growing in symbiosis with *Amœba dysenteriæ* and a number of unidentified bacteria.

Bacillus C.—An acid-fast bacillus cultivated from the nodules in the ears of a leprous patient and growing in symbiosis with amœbæ and a number of unidentified bacteria.

Bacillus D.—An acid-fast bacillus cultivated from the nodules in the ears of a leprous patient and growing in symbiosis with *Amœba dysenteriæ* and a number of unidentified bacteria.

Bacillus E.—An acid-fast bacillus cultivated from the nodules in the ears of leprous patients and growing in sympiosis with *Amœba dysenteriæ* and a mixed variety of bacteria.

Bacillus F.—An acid-fast bacillus cultivated from the spleen of a leper and growing in symbiosis with an amœba isolated from water, and with the cholera vibrio, obtained in pure culture and transplanted repeatedly on various culture media.

Bacillus G.—An acid-fast bacillus cultivated from a nodule in the thigh of a leprous subject and growing in symbiosis with a water amœba and cholera vibrio and in pure culture.

ANIMAL EXPERIMENTS.

Monkey No. 4440 (*Cynomulgus philippinensis* Geoff.) was inoculated subcutaneously with a suspension of four loops of a ten-day culture of Bacillus A, *Amœba dysenteriæ* and a variety of unidentified bacteria.

The animal died three months later and the autopsy showed no evidence of leprosy or of tubercular lesions.

Guinea pig No. 4484 was inoculated in the peritoneal cavity with one loop of a ten-day growth of Bacillus A, *Amœba dysenteriæ* and symbiotic bacteria. The animal died five weeks later from lobar pneumonia. The autopsy showed no evidence of leprosy, or tuberculosis.

Guinea pig No. 4485 was inoculated subcutaneously with a suspension of one loop of a ten-day culture of Bacillus A, *Amœba dysenteriæ* and symbiotic bacteria. Five weeks later it was found in a dying condition and was chloroformed. Autopsy showed hæmorrhagic areas located on the extremities, chest and abdomen. There was markedly œdematous tissue surrounding the joints. The heart was covered with small petechial hæmorrhages. All the vessels were injected. The lungs were

normal in consistency and no tubercles were present. All the glands were swollen and some, especially the mesenteric and inguinal, were hæmorrhagic.

Cultures made from the organs were negative.

Guinea pig No. 4486 was inoculated in the peritoneal cavity with a suspension of one loop of acid-fast Bacillus E, *Amœba dysenteriæ* and symbiotic bacteria. The animal died five weeks later. The autopsy findings were similar to those described in guinea pig No. 4485.

Guinea pig No. 4487 was inoculated in the same manner as the previous animal. The animal died six weeks later with similar lesions. There was no evidence of tuberculosis. Cultures made from all the organs remained sterile.

Guinea pig No. 4482 was inoculated in the peritoneal cavity with a suspension of one loop of a ten-day culture of Bacillus F in symbiosis with the cholera vibrio. The animal died one month later. There were severe hæmorrhages in all the organs. There was no evidence of tuberculosis.

Guinea pig No. 4483 was inoculated subcutaneously with one loop of a ten-day culture of Bacillus F and cholera vibrio. The animal died five weeks later with similar lesions. There was no evidence of tuberculosis. Cultures made from all the organs remained sterile.

The foregoing animal inoculations, while giving us little information in themselves as to the pathogenicity of the acid-fast organism, serve to separate the latter from the tubercle bacillus. In none of the injected animals were lesions found that resembled those of tuberculosis. The following animal experiments were made with one of the pure cultures of the acid-fast bacillus:

Guinea pig No. 4596 was inoculated subcutaneously with one loop of a ten-day pure culture of Bacillus F, suspended in 1 cubic centimeter of salt solution. After one week, an indurated nodular thickening appeared at the site of inoculation. This thickening and induration gradually spread and in four weeks the whole abdominal surface was involved. The hair of the skin surface corresponding with the lesion just described *fell out,* and before the exitus the animal became greatly emaciated; it died four weeks after the inoculation. Autopsy showed a marked thickening of the subcutaneous tissues of the abdominal wall, in some parts measuring 2 millimeters in thickness. There was hæmorrhagic infiltration of the muscular tissue as well as around the joints of the extremities. The abdominal cavity contained no fluid. The heart was slightly dilated. The lungs appeared normal. The spleen was enlarged to twice its normal size and was firm in consistency. Cut section showed an increase of fibrous connective tissue. The liver was enlarged and congested, presenting a dark, nearly black color. The kidneys showed some cloudy swelling. The stomach and intestines ap-

peared normal. Cultures made from the subcutaneous lesions were negative. Smears made from the lesions and stained by the acid-fast method showed the presence of large numbers of acid-fast bacilli, the majority of which were intracellular.

A portion of the nodule was placed in Zenker's fluid, hardened in the usual manner in alcohol and embedded in paraffine. The cut sections cut at four microns thickness were stained by a modified Borrel method, and also with eosin, followed by Unna's alkaline methylene blue.

The section includes muscular and subcutaneous tissue and nerve bundles. The nerves, and for the greater part the muscle fiber, show no abnormalities. The usual fibrous subcutaneous tissue has been replaced by richly cellular granulation tissue, consisting almost exclusively of irregularly spindle-shaped, young connective tissue cells. Near the cutaneous border of the section is found an oval-shaped, well-defined area consisting chiefly of leucocytes and necrotic tissue débris. In the rest of the section, leucocytes are present in very small numbers. In many of the cells of this tissue are found numerous short bacilli, some lying singly, but usually in dense masses in the protoplasm. These bacilli are stained deeply with the magenta in the Borrel sections and with methylene blue in the eosin-methylene blue preparations. In a few places the granulation tissue has involved the muscle bundles, and in this situation cells containing the bacteria are seen. Cells containing two or three distinct nuclei are found and in a few sections typical giant cells are seen with marginal nuclei. The blood vessels in the section contain a relatively large number of leucocytes.

The section when stained with carbol-fuchsin showed the presence of acid-fast bacilli corresponding in number with those found in the section just described.

Guinea-pigs Nos. 4597 to 4598 and 4599 were inoculated in a similar manner to Guinea-pig No. 4596. All died in the fourth week with the same symptoms and similar anatomical changes.

Cultures were made from the organs of these animals on media containing amœbæ and the cholera vibrio and on various laboratory media. In one instance only was the acid-fast microörganism reclaimed. The growth in this case occurred on the media containing the amœba and the cholera vibrio.

Guinea-pigs Nos. 4600, 4601, 4602 and 4603 were inoculated intraperitoneally with one loop of a ten-day culture of Bacillus F suspended in 1 cubic centimeter of salt solution. All the animals died during the fourth week. The autopsies showed marked hæmorrhages in the deep muscular tissues. The spleen was greatly enlarged in all the animals. There was severe congestion of the liver. There were no tubercles present and no cutaneous lesions. Cultures made from the

organs on media containing amœbæ and cholera vibrios and on ordinary laboratory media were negative.

White rats Nos. 4603, 4604 and 4605 were inoculated subcutaneously with a suspension of Bacillus F, lesions were produced on the site of inoculation consisting of a small, indurated nodule which disappeared after three weeks. Microscopical preparations made from the scrapings of the nodules and stained by the Ziehl-Neelson method showed large numbers of acid-fast bacilli. Cultures made from the nodules on media containing amœbæ and cholera vibrios and ordinary laboratory media were negative.

Twelve monkeys were inoculated subcutaneously and intraperitoneally with various amounts of Bacillus F. At the present time, three months later, there is no evidence of external lesions.

Upon failing to recover the bacillus from the internal organs of these experimental animals, a series of guinea pigs was inoculated subcutaneously with the splenic pulp of guinea pigs Nos. 4597 and 4598. Altogether six animals were inoculated. They died three weeks later with the same symptoms as those inoculated with the culture of the microörganisms. Cultures from the organs in these animals also remained sterile, and smear preparations examined microscopically showed no acid-fast bacilli. The splenic pulp of these animals inoculated subcutaneously into a third series produced identical lesions with fatal results. Cultures made from the organs remained sterile and no acid-fast bacilli were found in the smears.

DISCUSSION OF RESULTS.

Attempts have been made by me to cultivate the leprosy bacillus from the spleens of lepers and from cutaneous nodules in cases of leprosy in ten instances. In eight out of the ten instances an acid-fast bacillus was observed to multiply in successive transplants of the cultures of amœbæ to which the leprosy material had been added. Control plates of the amœba cultures without the addition of leprosy material showed no acid-fast organisms present. By heating such an amœba-cholera-leprosy culture a half hour at 60° C. and incubating, isolated colonies of the leprosy bacillus were obtained which grew readily in pure culture when transplanted to the ordinary laboratory media (nutrient agar, bouillon, coagulated egg medium, etc.).

Guinea pigs inoculated subcutaneously with the pure culture developed in some instances lesions at the site of inoculation which bear a certain resemblance to the leprous lesions of man, both macroscopically and when sectioned and examined under the microscope. The acid-fast organisms were found at the site of inoculation and in some instances also in the spleen. In one instance the acid-fast organism was

cultivated from a lesion in the guinea pig. These guinea pigs, as well as others which received intraperitoneal injections, invariably died during the third or fourth week after inoculation.

Inoculations into monkeys have yielded as yet only negative results.

It seems strange, at first thought, that, although the first culture of the leprosy bacillus on artificial media is attended with so much difficulty, it should grow so readily in subsequent transplants; but the tubercle bacillus often acts in a strikingly similar manner, only one or two tubes out of twenty or thirty yielding a growth from tuberculous tissue, whereas subcultures from this growth all develop luxuriantly on various media.

The majority of the lepers from whom the material for these cultures was obtained, had tuberculous lesions in their lungs, but the tissues used for the cultures showed no evidence of tuberculosis. The animal inoculations described in the text prove that the acid-fast organism cultivated by me is not the tubercle bacillus.

SUMMARY.

1. The leprosy bacillus was first cultivated from leprosy material in symbiosis with other unidentified bacteria and amœbæ and later from other cases in symbiosis with amœbæ and the cholera vibrio.

2. By heating a symbiotic culture of amœbæ, cholera and leprosy for a half hour at 60° C. and incubating, the leprosy bacillus was obtained in pure culture.

3. The leprosy bacillus isolated in this manner grows readily on the ordinary laboratory culture media.

4. The bacillus is pathogenic for guinea pigs, subcutaneous inoculations having caused lesions which macroscopically and microscopically resemble the leprous lesions of human subjects.

ILLUSTRATIONS.

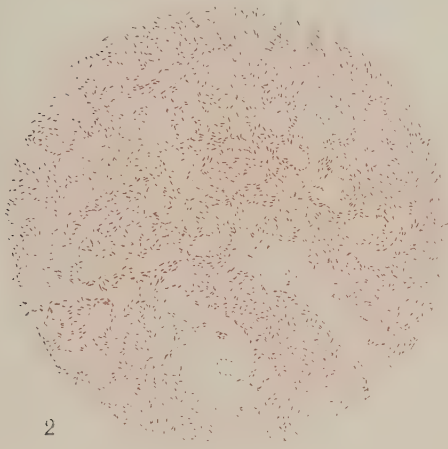
Plate I.

Fig. 1. Leprosy F. From pure culture in glycerine-bouillon.
2. Pure culture of Leprosy F from glycerine-agar.
3. Amœba with cholera vibrio and leprosy bacillus G. Second transplant from primary culture.
4. Amœba with cholera vibrio and leprosy bacillus C. Second transplant from primary culture.
5. Smear from early lesion in guinea pig following a subcutaneous injection of a pure culture of leprosy bacillus F.
6. Smear from leprous nodule in liver of human patient.

All of these preparations were stained with carbol-fuchsin and decolorized with Gabbet's stain.

Plate II.

Fig. 1. Smear from an early skin lesion of a guinea pig inoculated with a leprosy culture. Stained with carbol-fuchsin and decolorized with Gabbet's stain.
2. Amœba with cholera vibrio and leprosy bacillus C. Second transplant from primary culture.
3. Culture G. From pure culture of leprosy bacilli on glycerine-agar. Stained with carbol-fuchsin and decolorized with Gabbet's stain.
4. Culture F. From pure culture of leprosy bacilli on glycerine-agar. Stained with carbol-fuchsin and decolorized with Gabbet's stain.
5. Culture F. From pure culture of leprosy bacilli, in glycerine-bouillon. Stained with carbol-fuchsin and decolorized with Gabbet's stain.
6. Culture G. Leprosy bacilli with amœbæ and cholera vibrios. Stained with carbol-fuchsin and decolorized with Gabbet's stain.
7. Culture F. From pure culture of leprosy bacilli in glucose-bouillon. Two weeks' growth.
8. Culture F. From pure culture of leprosy bacilli on glycerine-agar. Two weeks' growth.



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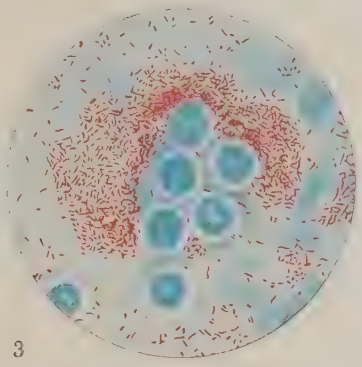

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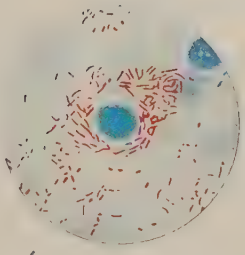

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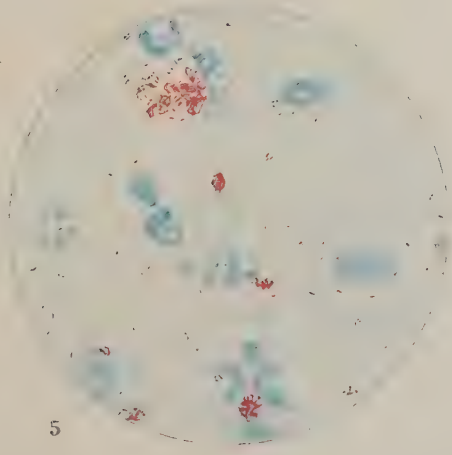

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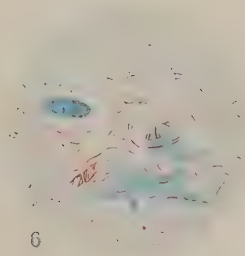

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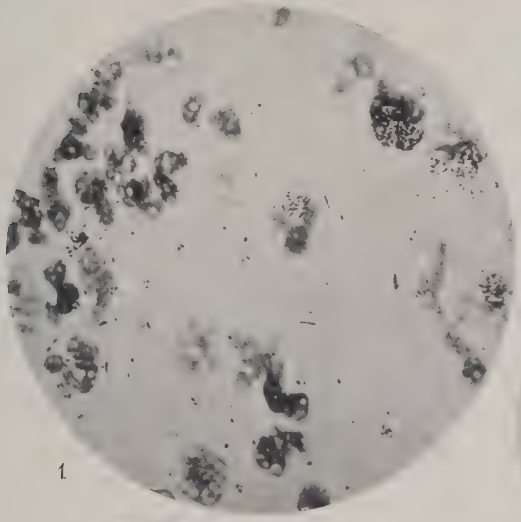
PLATE I.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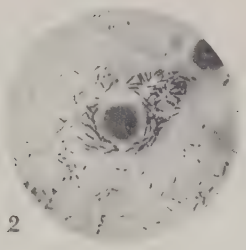
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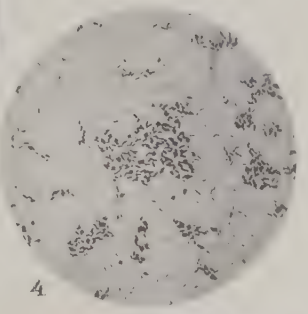
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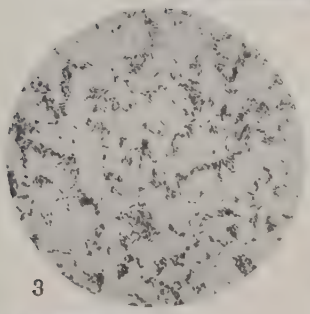
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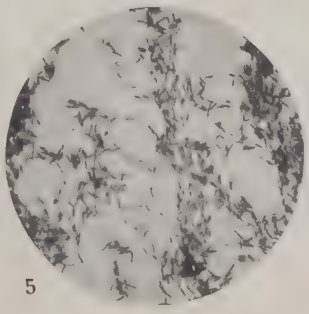
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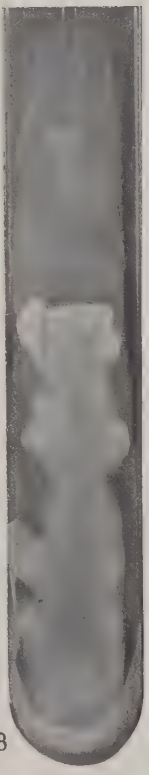
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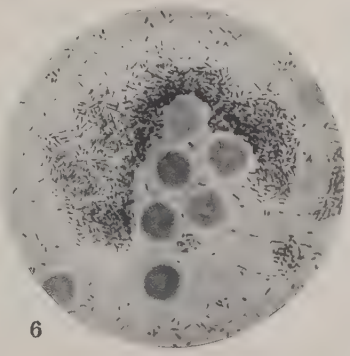
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PLATE II.

diarrhœa continued, the patient died. The following brief extracts are taken from the protocol:

Cause of death: Chronic endocarditis and acute purulent pericarditis. *Staphylococcus aureus* was isolated from the purulent exudate. The lungs show a number of areas of broncho-pneumonia. The intestine appears normal until the cæcum and colon are reached. Very numerous small erosions and ulcers may be observed in the mucosa scattered throughout almost the entire length of the large intestine, but more marked in the region of the cæcum and ascending colon. These appear to be of recent origin; none of them measure more than 1.0 centimeter in diameter. Their situation seems to bear no relation to the mesenteric attachment. They are more or less irregular in outline. Some are slightly undermined. There is little indication of acute inflammatory change about their bases or margins which are only slightly reddened. Many are merely erosions extending in depth scarcely through the mucosa; others apparently extend into the submucosa.

Scrapings from these lesions examined microscopically show the presence of the *Balantidium coli* in large numbers. No other protozoa such as *amœbæ* or *flagellata* were present.

The histological examination of sections of these ulcers showed the organisms lying free on the surfaces of the ulcers and invading the adjacent mucosa and muscularis mucosa, and occasionally reaching to the submucosa, where in a few instances they were observed within the blood vessels. The parasites were sometimes found in the submucosa at considerable distances from the ulcerations, and spaces and sinuses were seen in the tissue which had apparently been made by the passage of the parasites through it.

This case is evidently worthy of report for the reason that it has furnished information regarding the production of the very early intestinal lesions caused by this parasite. As Strong has pointed out in his earlier studies.[2]

"Whether *Balantidium coli* is capable of producing a primary erosion in the intestine has not been conclusively demonstrated. However, if such erosion of the mucosa exists from any cause, the parasite is certainly capable of continuing the process and of modifying and producing in connection with the bacteria which accompany it more or less characteristic pathologic lesions."

A study of the sections from these early ulcerations gives undoubted proof of this latter fact and from the spaces and sinuses left in the trail of this parasite in the submucosa, its mechanical injury to the tissue may be observed.

This case also conclusively demonstrates the fact that the *Balantidium coli* is not merely a secondary invader of chronic ulcerations which

[2] *Pub. P. I. Bur. Sci., Biolog. Lab.* (1904), No. 26, 1–77.

owe their origin to other organisms, for example to tubercle bacilli or to *amœbæ*.

The early ulcerations of the intestine are shown in Plate I.

Case II.—Male Filipino, who entered the tropical medical service of Doctor Strong with the following complaint: Severe diarrhœa, fever, cough, general weakness.

The patient on admission was in such a weak condition that all questions as to previous history, illness, habits, etc., were answered very unsatisfactorily. The following extracts are taken from the author's clinical history: The patient is very pale and somewhat emaciated. Glands of the groin somewhat enlarged, the other superficial lymphatic structures normal. Muscular power greatly diminished. The tongue is heavily furred and the breath is foul. He has no appetite. He expectorates large quantities of mucus semifluid sputum.

The pulse is weak, varying from 120 to 150 per minute, the tension is very low. On examination of the heart the apical impulse can not be seen or felt. The precordial area is hyperresonant and the heart's boundaries can not be sharply determined. On ausculation the heart sounds are weak and distant, but clear.

The respirations are shallow and rapid. Deep inspiration causes pain on both sides of the chest. The thorax on both sides generally is hyperresonant and on ausculation a few coarse râles may be heard at the bases of both lungs posteriorly.

The liver is not enlarged and the spleen can not be palpated. The abdomen is tense, and pressure, particularly over the region of the colon, causes severe pain.

During the time he remained in the hospital, diarrhœa was present, but at no time was it very acute; the number of bowel movements varied usually between five and ten in twenty-four hours. The stools, however, were always semifluid and contained much mucus and blood. The latter occasionally was passed in the form of clots. The temperature was usually normal in the morning, rising to 38° or 39° C. in the evening.

Twice during the course of his illness severe hæmorrhages from the bowels occurred. Immediately after the second hæmorrhage, the patient sank into a state of collapse, and died shortly afterwards.

Laboratory examinations: A blood smear showed no malarial parasites and a Widal test and blood culture also proved negative. The counts of the red blood corpuscles varied from 3,500,000 to 2,500,000. The leucocytes averaged 9,500. A differential count showed a moderate increase in the mononuclear elements and 8 per cent of eosinophiles.

The sputum was negative for tubercle bacilli and for parasites.

The microscopic examination of the fæces showed the presence of the ova of *Ascaris lumbricoides, Uncinaria duodenale* and large numbers

of the *Balantidium coli.* These protozoa were especially numerous in the flakes of mucus scattered through the specimen.

The urine was highly colored and of high specific gravity. It contained much albumen, hyaline and granular casts and cylindroids. No parasites were present.

Necropsy: Performed four hours after death by the author.

Body extremely emaciated, subcutaneous fat very scanty. *Rigor mortis* is absent.

Heart and pericardial cavity: There is a small amount of clear, straw-colored fluid present in the pericardial cavity. Both serous layers are smooth and glistening.

The heart is small and the surface pale in color. Some fluid blood is present in the right ventricle. On section, the muscle is seen to be thin and pale in color. Valves, and coronary arteries, and aorta, apparently normal.

Lungs and pleural cavity: The left lung is pale and emphysematous and is adherent to the pleura by old fibrous adhesions and can with difficulty be separated from it. No nodules can be felt in the lung substance, and on section none can be seen.

The right lung is emphysematous above and in the lower lobe is somewhat congested. The pleuræ are adherent and can not be separated without tearing the lung tissue.

The bronchi contain considerable mucus; the bronchial glands are normal in size and consistency.

Abdominal cavity: On opening the peritoneal cavity the appearance of the peritoneum is not glistening but is slightly dull. This appearance is more marked in the pelvic portion. In the pelvis, the mesentery is somewhat matted together. No nodules are seen on the peritoneum.

There is little omental fat. Some of the mesenteric lymph glands are enlarged, one or two of them being quite hard, and as large as a pigeon's egg; others situated along the line of the mesenteric attachment of the colon on cut section show inflammatory change and softening. The appendix appears normal. In the pelvis, free fæces are present.

The spleen is slightly enlarged and pale. On section the malpighian bodies are clearly visible.

The kidneys: The capsules are somewhat adherent. On section the kidneys appear pale and cloudy; the striations are irregular and very indistinct.

Liver: Not enlarged; of a dark red color and having a glistening surface. On section it appears normal.

The *pancreas* appears normal.

Intestines: The small intestine is practically normal to within a few centimeters of the cæcum, where definite ulcers are situated which have an irregular shape. Some have tags of necrotic tissue attached to the bases and undermined edges.

The walls of the cæcum are greatly thickened and are riddled with ulcerations. Many of these have healed, others show evidences of a very active process. In the fundus of the cæcum is an ulcer which has

perforated into the peritoneal cavity. Other ulcers are found scattered throughout the colon.

The situation of the ulcers bears no definite relation to the mesenteric attachment. They vary in shape, some being oval, others round, and nearly all have ragged edges. The early ulcerations are clean, shallow, and not injected, while the older ones are irregular in outline with their bases covered with mucus and necrotic tissue. Scrapings made from the bases of these ulcers show the presence of large numbers of the *Balantidium coli*. No amœbæ or other protozoa are observed.

The rectum is injected, but free from ulceration.

HISTOLOGICAL EXAMINATION.

The results of the histologic examination of sections of the large intestine and of the mesocolic lymph glands only will be described.

The parasites were found in all the sections of the ulcers examined, being situated both in the mucosa and submucosa. The blood vessels were dilated and contained many parasites. Large numbers of them were also found in the muscular layers. Some of the parasites showed division forms or elongation of the nucleus.

There appeared to be but moderate round-celled infiltration in the deeper layers of the intestine, except where there were several parasites grouped together. Single parasites were found in the tissues far away from the ulcerations.

Sections of an enlarged mesocolic lymph gland removed from the neighborhood of the mesenteric attachment to the intestine showed numerous single parasites which appeared scattered through the lymph sinuses of the gland tissue and situated within the blood vessels. The parasites were not found in groups anywhere in the gland.

Plate IV fig. 1 shows eight parasites in one field. The nuclei of some of the parasites are not visible. There are areas of necrosis in the glandular tissue. A small area of necrosis is shown in Plate IV fig. 2. The parasites are not usually situated in these areas of necrosis, but sometimes single parasites are found nearby. During life the parasites are almost constantly in motion.

Sections of the intestinal ulcers and of the glands, stained by the Ziehl-Neelson method, showed no tubercles or tubercle bacilli.

An examination of a portion of a fresh gland crushed out on a slide and stained in the same way was also negative for tubercle bacilli.

SUMMARY.

Case I shows particularly the earliest stages in the ulcerative process caused by the *Balantidium coli*. (See Plate I.) Case II demonstrates particularly (see Plate II) the later stages of ulceration and also that this process can proceed to the stage of perforation with resulting

general peritonitis and death.[3] This case, however, never reached the stage of advanced general peritonitis, for at the time of perforation there occurred a severe hæmorrhage, the patient losing a liter or more of blood, and this, with the shock of the perforation, in his weakened condition caused almost immediate death.

In 1904, Strong [4] collected from the literature all the cases of *Balantidium coli* infection (127 in number) reported up to that time. In two of these cases death occurred from general peritonitis.

The presence of *Balantidium coli* in the mesenteric lymph glands in the second case reported in the present paper is peculiar to this case and has not been described before.

In Doctor Strong's series none of the cases showed abscess of the liver and only in one was the parasite reported as present in the sputum from a case with an indefinite diagnosis of abscess of the lung. The parasites are very frequently situated in the lymph spaces and in the blood vessels of the submucosa, but they do not appear to have caused inflammatory conditions elsewhere in the body. As has been stated, some of the mesocolic lymph glands in the second case showed areas of inflammatory change and of necrosis. On account of the size of the *Balantidium coli* wherever the parasite enters, the intestinal bacteria would also have an opportunity of entering and of causing inflammatory changes. However, bacteria were not definitely discovered in sections of these glands. In this connection it is interesting to note that Glaessner [5] in extracts of *Balantidium coli* in the fæces was able to demonstrate a very active diastase and a fairly strong hæmolytic substance, although the extracts were inert with respect to dilute albumen, peptone solution and to fibrin.

CONCLUSIONS.

I. The intestinal lesions in both of these cases seem to have been caused by *Balantidium coli.*

II. Death may result from perforation in severe *Balantidium coli* ulceration.

III. The presence of the parasite is not always limited to the walls of the intestine. The organism may invade the mesocolic intestinal lymphatic glands.

[3] Both specimens of the intestine from which these illustrations were prepared are at present in the museum of the Biological Laboratory of this Bureau.

[4] *Pub. P. I. Bur. Sci., Biolog. Lab.* (1904), **No. 26**, 1–77.

[5] *Centralbl. f. Bact. Orig.* (1909), **47**, 351.

ILLUSTRATIONS.

PLATE I. From Case I. Large intestine, *Balantidium coli* infection. Showing early lesions, consisting of numerous small erosions and ulcerations in the mucosa.

II. From Case II. Large intestine, *Balantidium coli* infection. Showing advanced ulcerative lesions.

III. Large intestine. Fig. 1. Numerous parasites in the submucosa. Fig. 2. Two parasites in a blood vessel in the submucosa.

IV. Lymphatic gland, showing area of necrosis. Fig. 1. Eight of the parasites may be seen distinctly in the photograph. Fig. 2. A single parasite lying near an area of necrosis.

V. Lymphatic gland. Fig. 1. Single parasite. Fig. 2. Four parasites may be observed lying in spaces in the lymphoid tissue about an area of necrosis.

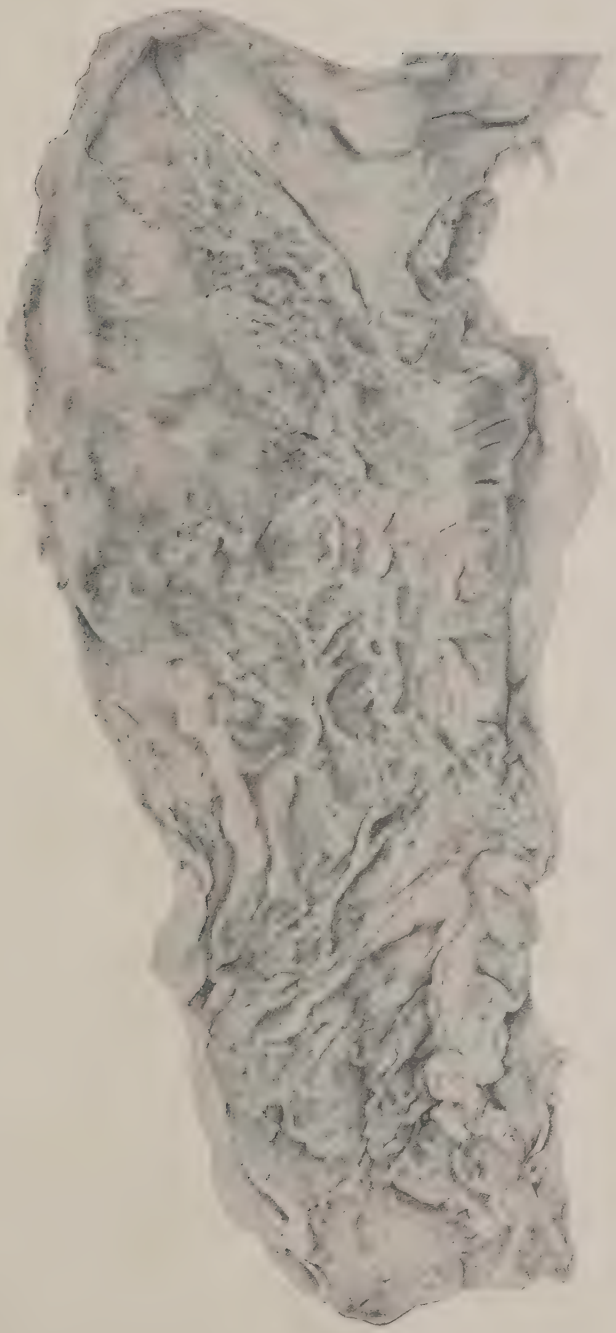


PLATE I.

PLATE II.

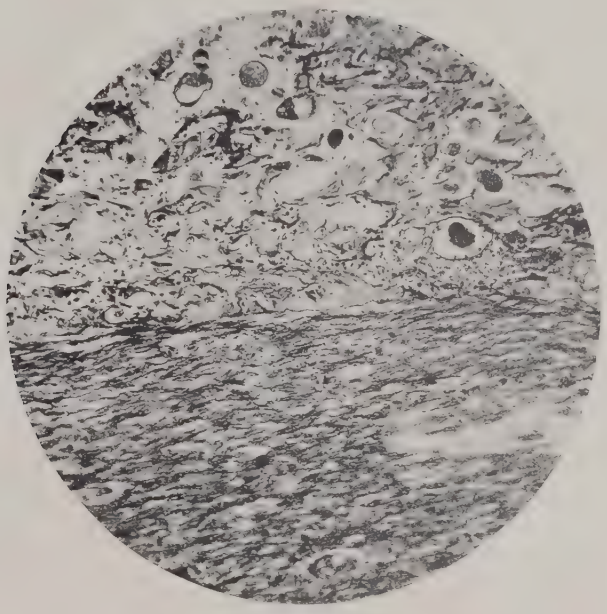


Fig. 1.

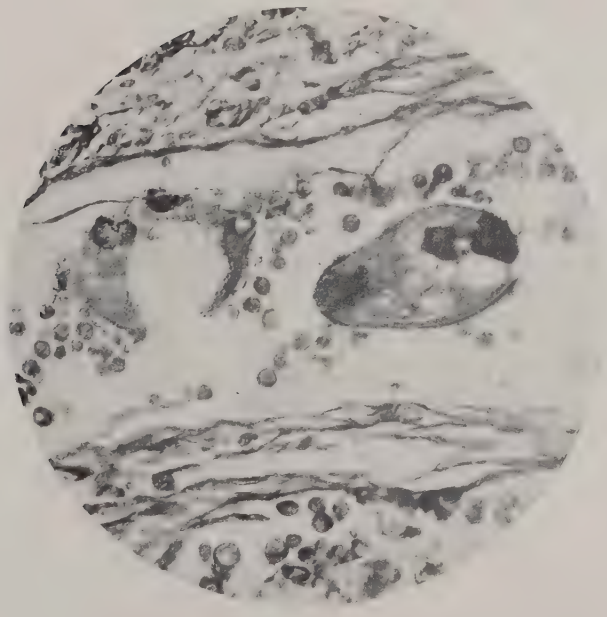


Fig. 2.

PLATE III.

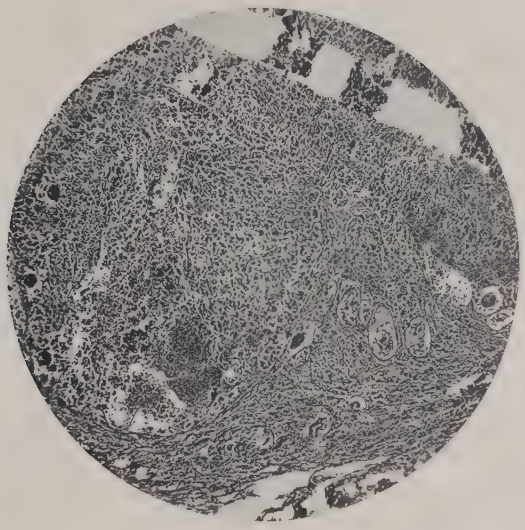


FIG. 1.

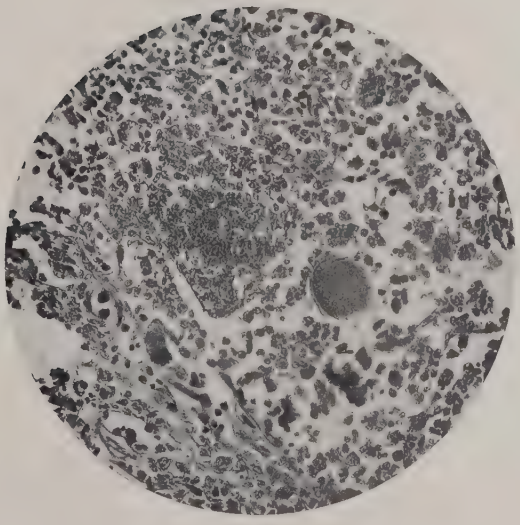


FIG. 2.

PLATE IV.

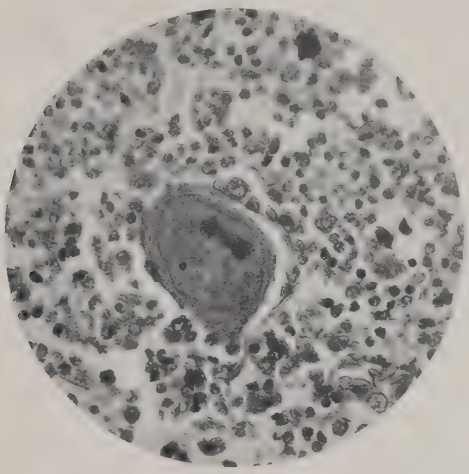


Fig. 1.

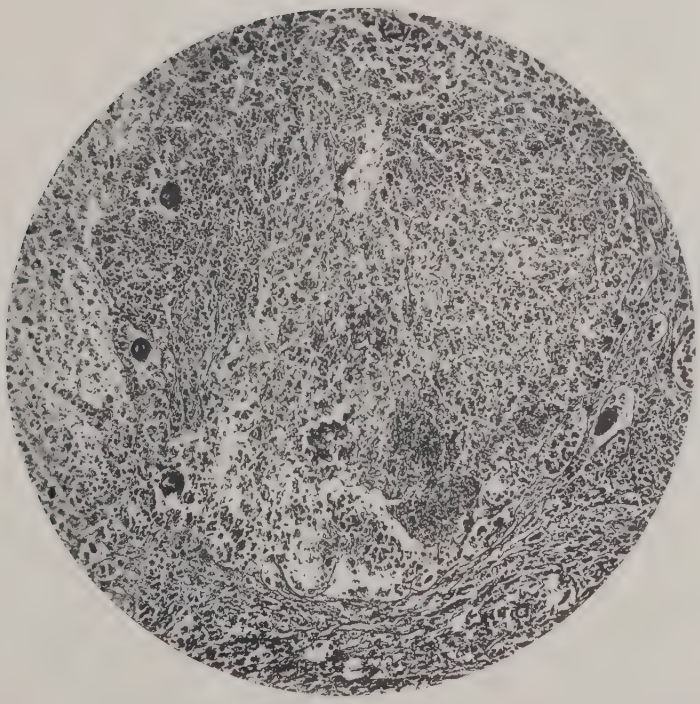


Fig. 2.

PLATE V.

THE DIFFERENCE IN SUSCEPTIBILITY TO CATTLE PLAGUE ENCOUNTERED AMONG CATTLE AND CARABAOS.

By E. H. Ruediger.

(*From the Serum Section, Biological Laboratory, Bureau of Science, Manila, P. I.*)

Perhaps the most perplexing problem the worker with cattle plague meets is the dosage of anticattle-plague serum necessary for protective and curative purposes.

Rogers,[1] working in India, prepared anticattle-plague serum and determined its immunizing value on seven head of plains cattle which had never suffered from cattle plague. They received 10, 10, 15, 15, 20, 25 and 30 cubic centimeters of serum, respectively, per 300 kilograms of weight, and 1 cubic centimeter of virulent blood. Three animals showed mild symptoms of plague, two showed moderate rise of temperature, and the remaining two did not react at all. The reactions were entirely independent of the quantity of serum the animals had received, showing a difference in susceptibility.

In his second test, Rogers used 15, 20, 25 and 35 cubic centimeters of serum, respectively, per 300 kilograms of weight on four animals. The first three reacted well. Eleven other cattle were given 20 cubic centimeters of serum and 1 cubic centimeter of virulent blood. Eight reacted moderately and three did not show any reaction.

Of the twenty-two head of plains cattle reported on by Rogers, eight did not react to inoculation with 1 cubic centimeter of virulent blood. From eight to ten days after the first inoculation, these eight animals received 10 cubic centimeters of virulent blood each, and in five of them this inoculation was followed by good reactions.

It is to be regretted that throughout this work Rogers did not use any control animals, for all or nearly all his animals might have had only a mild reaction without any serum.

Having found wide variations in susceptibility among cattle on the plains where cattle plague had been endemic and epidemic for many years, Rogers resorted to mountain cattle from districts that had never been visited by cattle plague, hoping that these would react uniformly to inoculation.

Doses of 10 cubic centimeters of serum per 300 kilograms of weight were worthless against 1 cubic centimeter of virulent blood. Of eight animals so inoculated, seven died of cattle plague. On increasing the quantity of serum to 40 cubic centimeters, 50 per cent, and with doses of 90 cubic centimeters, 66 per cent of the cattle were saved.

[1] *Ztschr. f. Hyg.* (1900), **35**, 59.

Five animals were inoculated with 0.5 cubic centimeter of virulent blood and were given 90 cubic centimeters of serum per 300 kilograms of weight. Four of them died of cattle plague and one showed no reaction whatever.

Stockman [2] inoculated fifty head of cattle in India with 4 cubic centimeters of virulent blood and gave them serum varying in quantity from 2 to 10 cubic centimeters. All lived. Nine control animals received 4 cubic centimeters of virulent blood and none of these died.

Eight buffaloes that received 4 cubic centimeters of virulent blood and from 1.8 to 10.2 cubic centimeters of serum, all lived. Four control buffaloes that had been inoculated with 4 cubic centimeters of virulent blood also lived.

Walker [3] found that in India the cattle on the plains or lowlands are far more resistant toward cattle plague than are the cattle on the mountains. Mountain cattle require eighteen times as much serum as do plains cattle. In a later report [4] Walker proposes the following plan for estimating the quantity of immune serum required to protect contact animals during an epidemic of cattle plague: If the death rate is 50 per cent or less, use the standard dose of serum; in an epidemic with a mortality of from 50 to 75 per cent, double the standard dose; if from 75 to 85 per cent, use eighteen times the standard dose of serum.

According to the experience of Daniels [5] with five different breeds of animals in India, Malay buffalo or cariboo (Kerbau) are highly susceptible to cattle plague; while Siamese cattle, Bali bullocks, Indian cattle and Indian water buffaloes are but slightly susceptible.

Kolle [6] makes the statement that in his experience 30 cubic centimeters was the most satisfactory quantity of serum used in the simultaneous method of immunization.

The following results obtained in testing the immunizing value of anticattle-plague serum at Manila may be of interest. Anticattle-plague serum is here prepared on a large number of animals and each lot of serum is a mixture of the serum derived from forty or fifty. In order to obtain serum of uniform quality, bleedings are arranged to include animals in all stages of the bleeding period. The serums here reported upon, with one exception, lot No. 810, may be considered of fairly uniform quality. The latter was obtained during an outbreak of foot and mouth disease among the serum animals. Inoculations and bleedings could not be arranged satisfactorily, and the immunizing value of this lot was lower than usual, but the test is of importance in showing the comparative susceptibility of native cattle and carabaos. The animals used in these tests were all in good health, they weighed about 200 kilograms—unless otherwise indicated—and the quantity of serum mentioned represents the dose per 100 kilograms weight of animal.

[2] *Journ. Comp. Path. and Ther.* (1903), **16**, 319.
[3] *Journ. Comp. Path. and Ther.* (1904), **17**, 326.
[4] *Journ. Trop. Vet. Science* (1908), **3**, 28.
[5] *Loc. cit.* (1907), **2**, 159.
[6] Handbuch der Technik und Methodik der Immunitätsforschung. Jena (1909). Zweiter Band, p. 595.

TEST 1.

Five bullocks, numbered 1, 2, 3, 4 and 5, natives of the Island of Sibuyan, received 0.5 cubic centimeter of virulent blood each. Animal No. 1 was used as control, Nos. 2, 3, 4 and 5 received 25, 50, 75 and 100 cubic centimeters of anticattle-plague serum, respectively.

TABLE 1.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	cc.	
1	0.5	None.	Died.
2	.5	25	Lived.
3	.5	50	Lived.
4	.5	75	Lived.
5	.5	100	Lived.

Table 1 shows the results obtained. All animals lived except No. 1, which was used as a control. (See charts 1 to 5.)

TEST 2.

On the 15th day of March, 1909, Sibuyan bullocks Nos. 6, 7, 8 and 9 were given 0.5 cubic centimeter of virulent blood each. Animal No. 6 was used as control, Nos. 7, 8 and 9 were given 25, 50 and 100 cubic centimeters of serum No. 767, respectively.

TABLE 2.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	cc.	
6	0.5	None.	Died.
7	.5	25	Died.
8	.5	50	Lived.
9	.5	100	Lived.

All animals reacted well after inoculation and, as is shown in Table 2, Nos. 6 and 7 died, Nos. 8 and 9 lived. (See charts 6 to 9.)

TEST 3.

Serum No. 810 was tested on five bullocks, Nos. 10, 11, 12, 13 and 14, which came from the Batanes group of islands. Each animal received 0.5 cubic centimeter of virulent blood. No. 10 acted as control and Nos. 11, 12, 13 and 14 received 50, 75, 100 and 125 cubic centimeters of serum, respectively.

TABLE 3.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
10	0.5	None.	Died.
11	.5	50	Died.
12	.5	75	Died.
13	.5	100	Died.
14	.5	125	Died.

As table 3 shows all the animals died succumbing to cattle plague. Serum No. 810 was below the average in immunizing value. (See charts 10 to 14.)

TEST 4.

On the 6th day of July, serum No. 810 was tested on eight native carabaos, Nos. 15 to 22. All received 0.5 cubic centimeter of virulent blood, No. 15 was used as control, and Nos. 16 to 22 received 75, 100, 100, 125, 125, 150, and 150 cubic centimeters of serum, respectively.

TABLE 4.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
15	0.5	None.	Died.
16	.5	75	Died.
17	.5	100	Lived.
18	.5	100	Lived.
19	.5	125	Lived.
20	.5	125	Lived.
21	.5	150	Lived.
22	.5	150	Lived.

All animals underwent good reactions and as is shown in Table 4, animals Nos. 15 and 16 died, Nos. 17 to 22, inclusive, lived. Carabaos appear to be more resistant than the Batanes cattle to the disease. (See charts 15 to 22.)

TEST 5.

Batanes bullocks Nos. 23, 24, 25, 26 and 27 received 0.5 cubic centimeter of virulent blood each. No. 23 was used as control and Nos. 24, 25, 26 and 27 received 25, 50, 75 and 100 cubic centimeters of serum No. 872.

TABLE 5.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
23	0.5	None.	Died.
24	.5	25	Died.
25	.5	50	Lived.
26	.5	75	Lived.
27	.5	100	Lived.

Table 5 shows the results obtained. Animals Nos. 23 and 24 died of cattle plague, Nos. 25 to 27, inclusive, underwent good reactions but recovered. (See charts 23 to 27.)

TEST 6.

Early in August, 1909, Doctor Gearhardt, of the Bureau of Agriculture, sent to the Biological Laboratory Bureau of Science several bottles of foreign anticattle-plague serum, and requested that its immunizing value be determined. According to directions on these bottles, 20 cubic centimeters of the serum given by the simultaneous method should have been sufficient to protect medium-sized animals against a fatal attack of cattle plague.

On the 15th day of August, five Batanes bullocks, Nos. 28 to 32, inclusive, were each inoculated with 0.5 cubic centimeter of virulent blood. Animal No. 28 was used as control, Nos. 29 to 32, inclusive, received 25, 50, 75 and 100 cubic centimeters of foreign serum, respectively.

TABLE 6.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
28	0.5	None.	Died.
29	.5	25	Died.
30	.5	50	Died.
31	.5	75	Died.
32	.5	100	Died.

The results obtained with the foreign serum are recorded in Table 6. All animals died of typical cattle plague despite the fact that animal No. 32 had received ten times the prescribed dose of serum. (See charts Nos. 28 to 32.)

TEST 7.

The foreign serum was now tested on Indo-China heifers Nos. 34, 35, 36, 37 and 38, controlled by Batanes bullock No. 33.

Animals Nos. 34, 35, 36, 37 and 38 received 5 cubic centimeters of virulent blood each and 20, 20, 40, 80 and 100 cubic centimeters of serum respectively. No. 33 received 0.5 cubic centimeter of virulent blood.

TABLE 7.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
33	0.5	None.	Died.
34	.5+5	20	Lived.
35	.5+5	20	Lived.
36	.5+5	40	Lived.
37	.5+5	80	Lived.
38	.5+5	100	Lived.

A glance at Table 7 shows that the control died and the others lived. Control animal No. 33 gave a good reaction for cattle plague and died on the twelfth day after inoculation. Of the five Indo-China heifers, only one, No. 35, gave a good reaction. On inspecting the temperature charts, it will be noticed that the dates of chart 33 do not correspond to those of charts 34, 35, 36, 37 and 38. A suitable control animal was not at hand when animals 34, 35, 36, 37 and 38 were inoculated. None having died, and only one having reacted well to the first inoculation, they were reinoculated with 5.0 cubic centimeter of virulent blood each, and the control animal, No. 33, received 0.5 cubic centimeter of virulent blood. (See charts 33 to 38.)

TEST 8.

Anticattle-plague serum No. 891, prepared in the Philippine Islands, was tested on Indo-China heifers, Nos. 40, 41, 42, 43 and 44, each weighing about 100 kilograms, controlled by Batanes bullock No. 39. All were inoculated with 0.5 cubic centimeter of virulent blood, and animals Nos. 40, 41, 42, 43 and 44 received 10, 20, 30, 40 and 50 cubic centimeters of serum.

TABLE 8.

No. of animal.	Quantity of V. B.	Quantity of serum.	Results.
	cc.	*cc.*	
39	0.5	None.	Died.
40	.5	10	Lived
41	.5	20	Lived
42	.5	30	Lived.
43	.5	40	Lived.
44	.5	50	Lived.

The results in Table 8 show that the control died and all the Indo China heifers lived. (See charts 39 to 44.)

TEST 9.

Indo-China heifers Nos. 46, 47, 48, 49 and 50, controlled by Batanes bullock No. 45, were tested for immunity toward cattle plague. Each animal was inoculated with 2 cubic centimeters of virulent peritoneal fluid (blood was not at hand).

TABLE 9.

No. of animal.	Quantity of fluid.	Results.
	cc.	
45	2	Died.
46	2	Lived.
47	2	Lived.
48	2	Lived
49	2	Lived.
50	2	Lived.

Table 9 shows the results obtained. The control died, while the Indo-China heifers lived; each, however, showed a moderated temperature reaction. (See charts 45 to 50.)

SUMMARY.

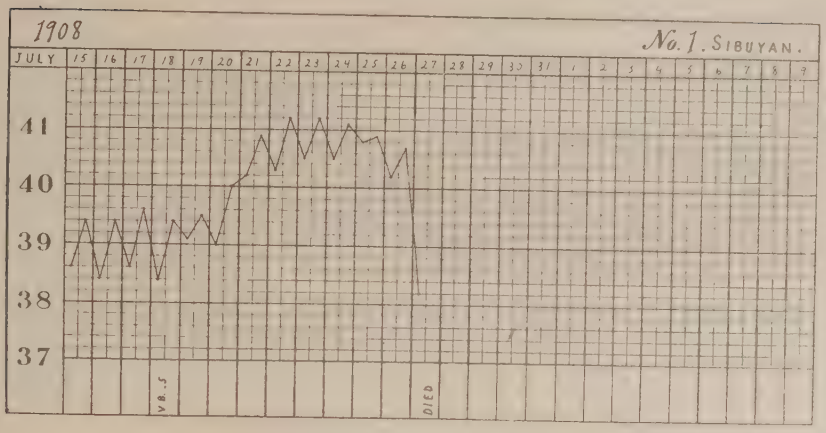
In summing up the results of other investigations and those reported above, it is found that cattle vary greatly in susceptibility to cattle plague. In India the cattle on the plains are resistant while those on the mountains are highly susceptible.

Philippine cattle from noninfected districts are highly susceptible and the carabao, as shown in Tests 3 and 4, appear to be more resistant than the Batanes cattle.

Cattle imported from Indo-China are highly resistant to cattle plague.

ILLUSTRATIONS.

Charts 1 to 50.


1908
No. 1. SIBUYAN.
JULY
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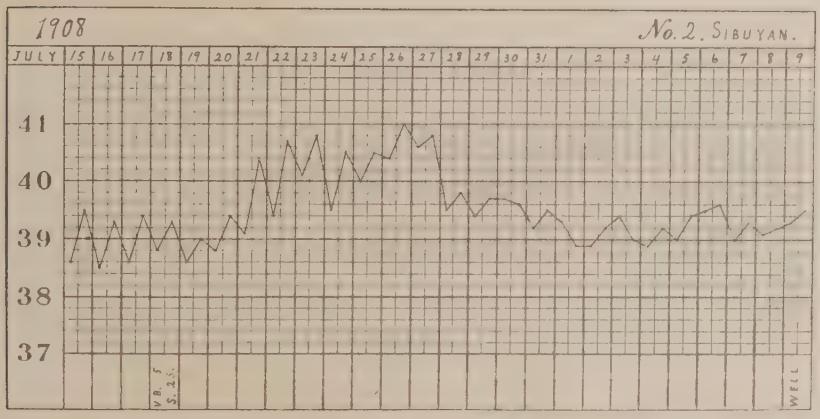
41
40
39
38
37
V.B.S
DIED


1908
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JULY
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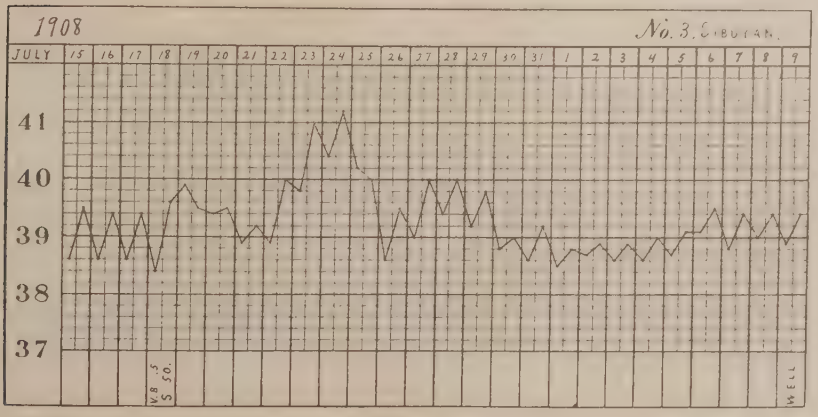
41
40
39
38
37
V.B.S
S.2.S.
WELL


1908
No. 3. SIBUYAN.
JULY
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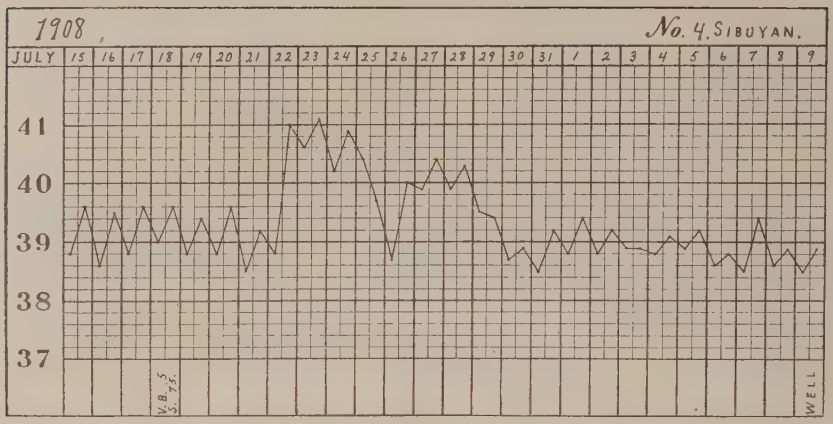
41
40
39
38
37
V.B.S
S.S.O.
WELL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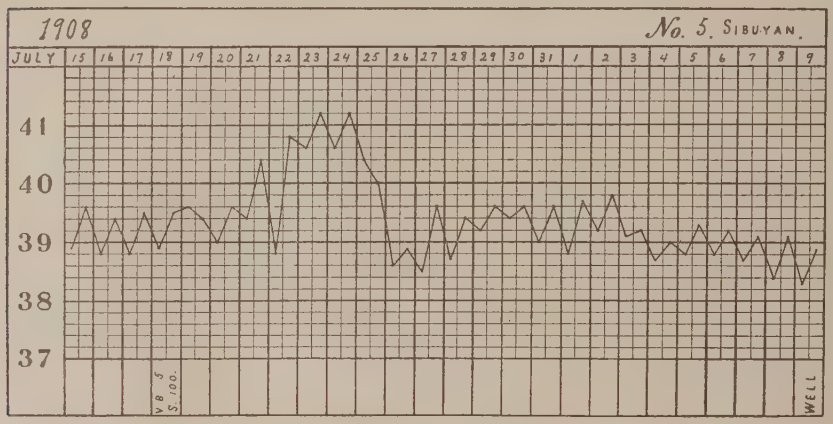
No. 4. SIBUYAN.
JULY 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9
41
40
39
38
37
V.B.S S.75.
WELL


1908
No. 5. SIBUYAN.
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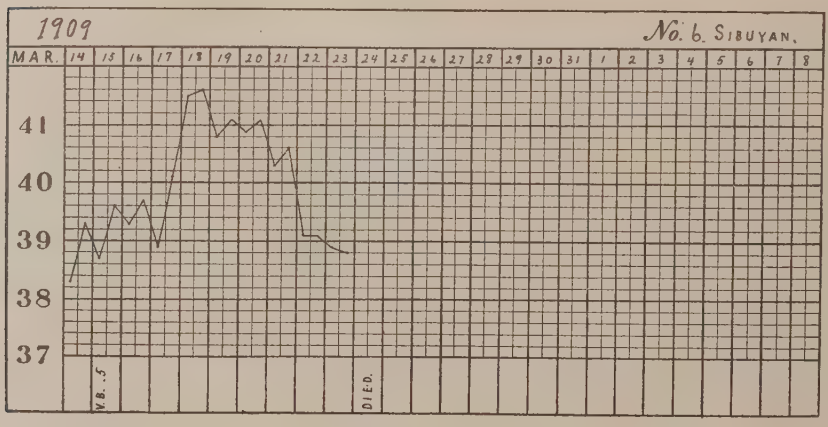
41
40
39
38
37
V.B.S S.100.
WELL


1909
No. 6. SIBUYAN.
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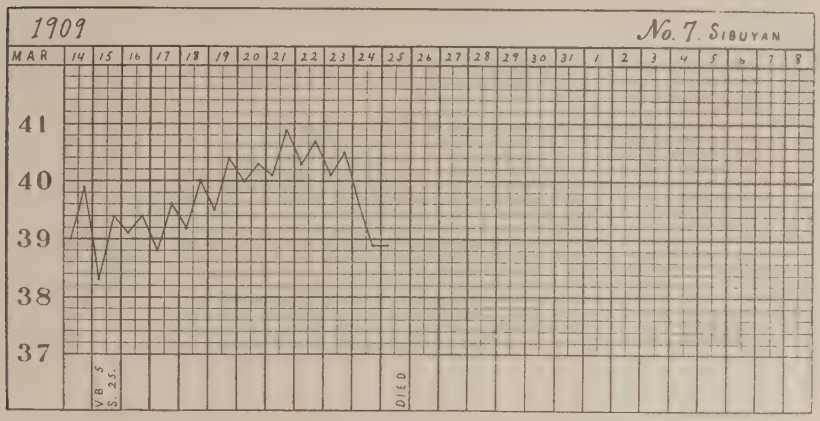
41
40
39
38
37
V.B..S
DIED.


1909
No. 7. Sibuyan
MAR
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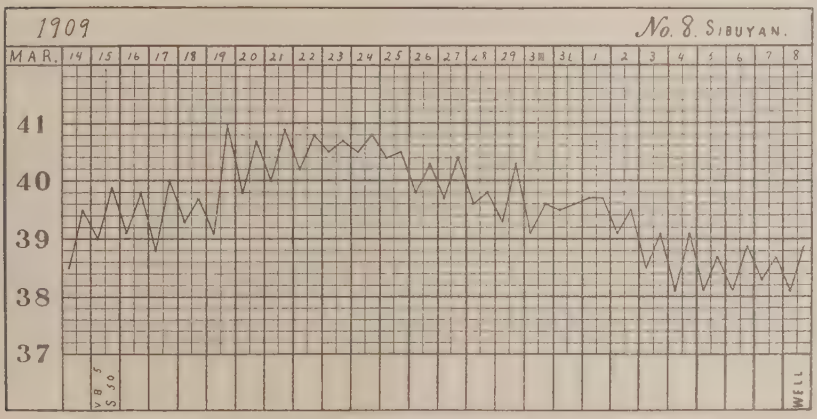
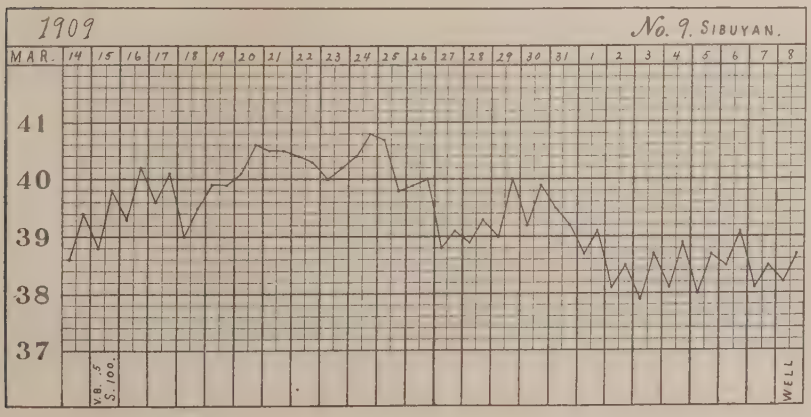
41
40
39
38
37
V.B. 5
S. 25.
DIED


1909
No. 8. Sibuyan.
MAR.
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8
41
40
39
38
37
V.B. 5
S. 50
WELL


1909
No. 9. Sibuyan.
MAR.
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41
40
39
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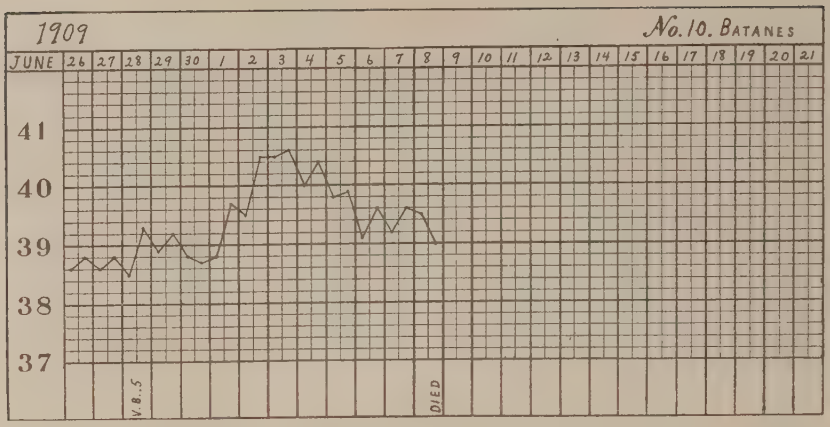
37
V.B. 5
S. 100.
WELL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No. 10. BATANES
JUNE 26 27 28 29 30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
41
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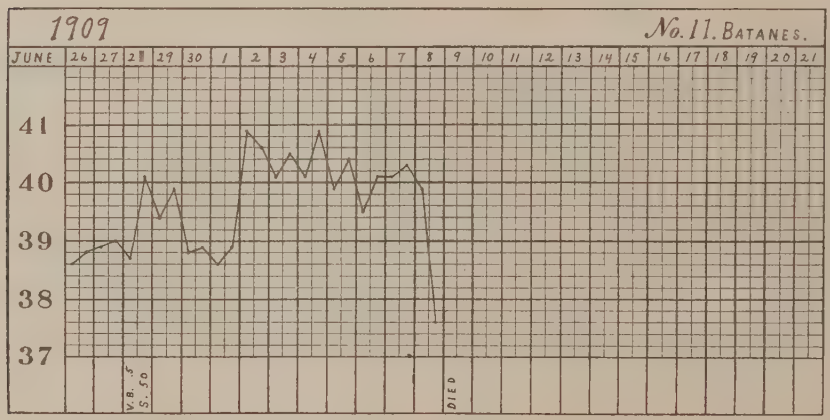
37
V.B. S
DIED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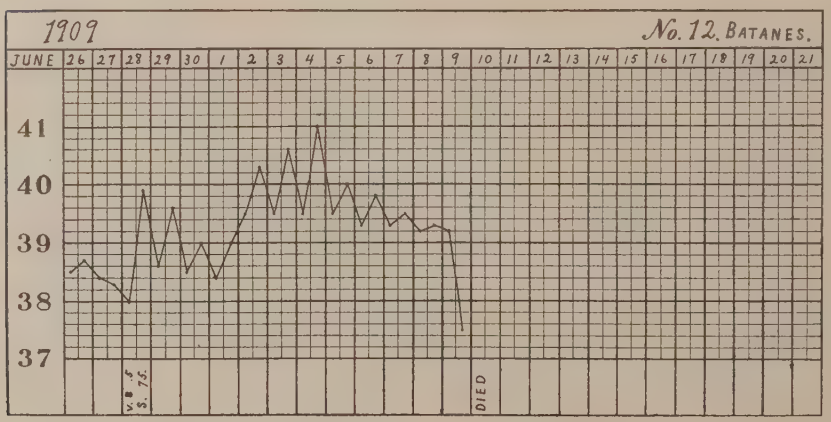
V.B. S
S. 50
DIED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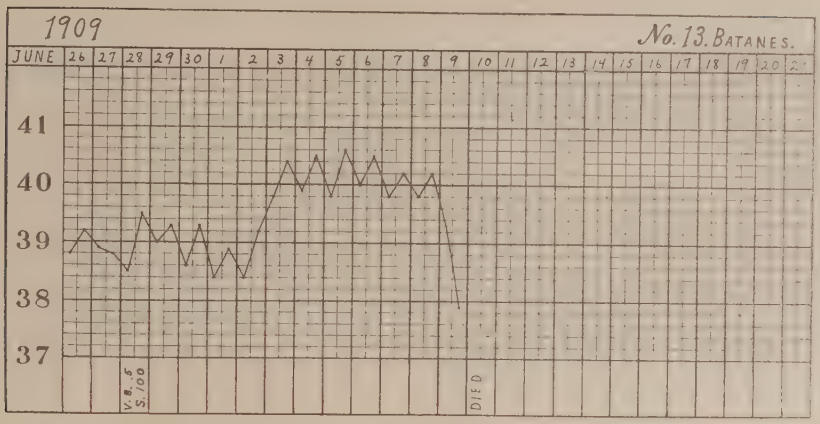
37
V.B. S
S. 75
DIED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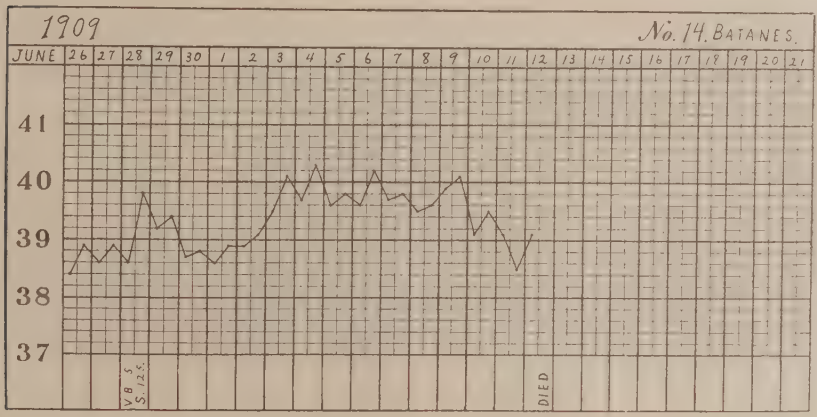
V.B.S S.100
DIED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JUNE
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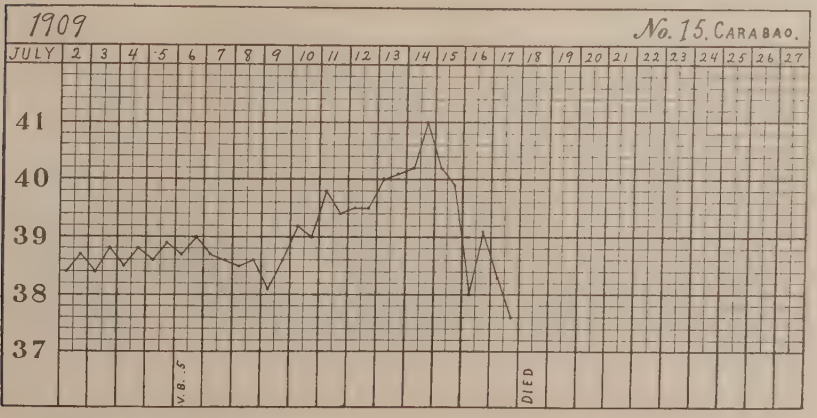
V.B.S S.12.5
DIED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No. 15. CARABAO.
JULY
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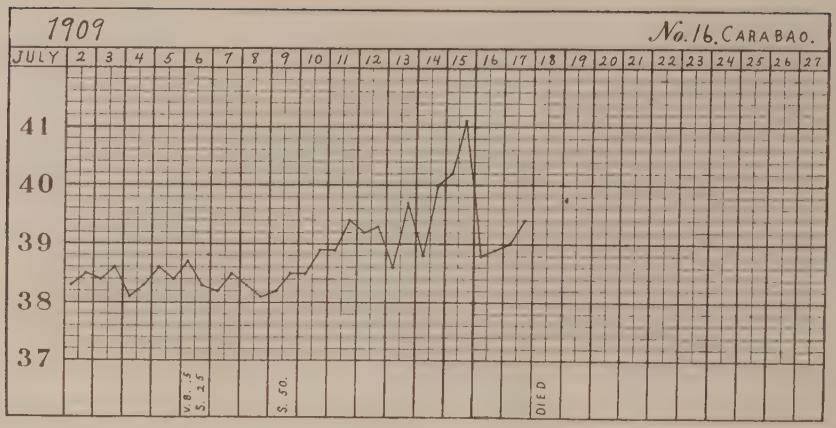
37
V.B.S
DIED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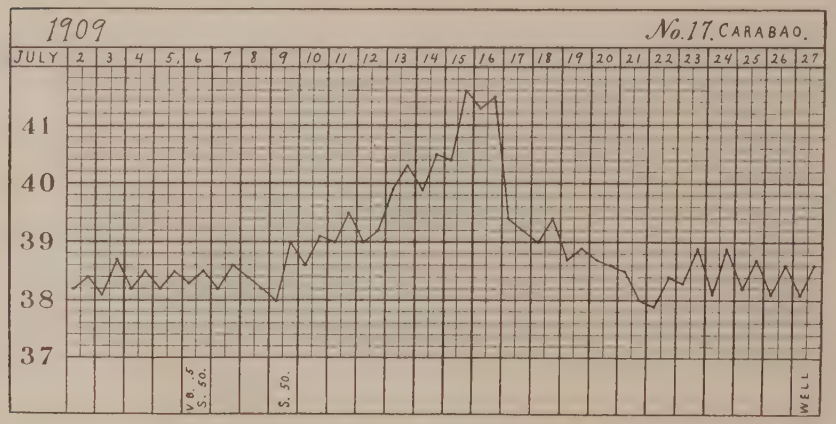
VB. .5
S. 25
S. 50.
DIED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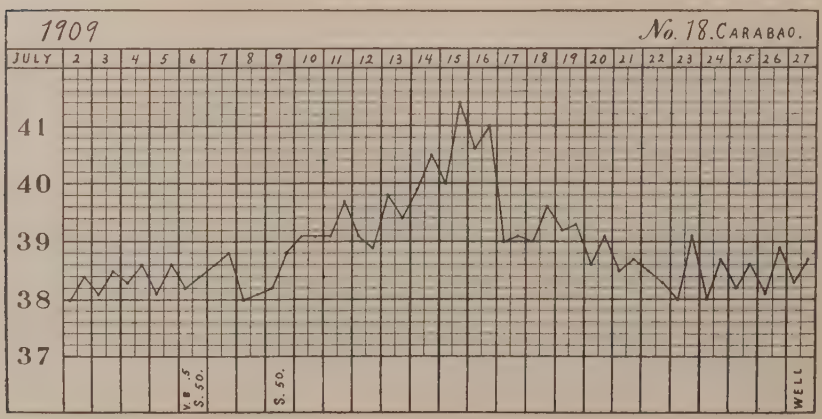
VB. .5
S. 50.
S. 50.
WELL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VB. .5
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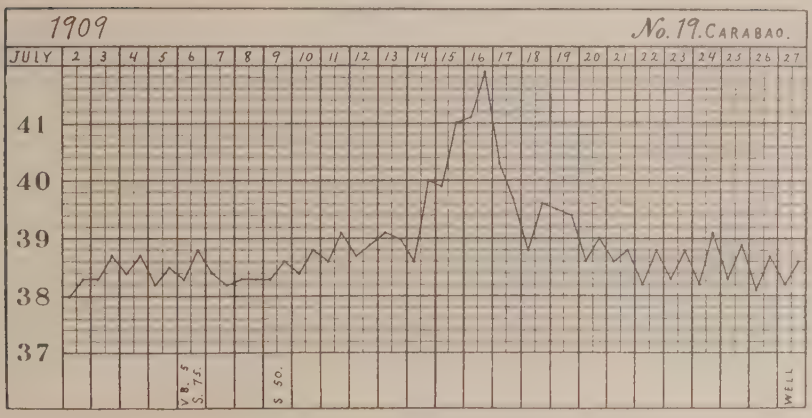
S. 50.
WELL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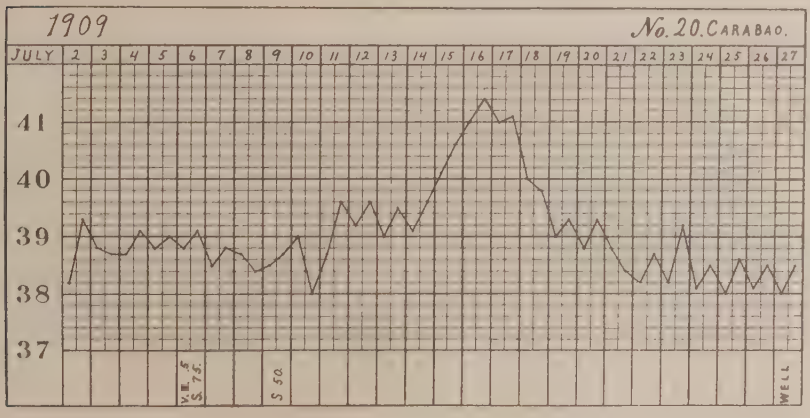
V.B.S S.75
S 50.
WELL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JULY 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
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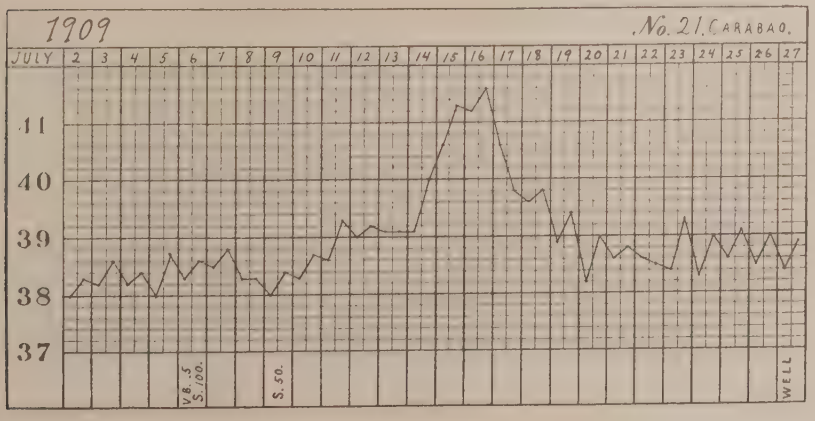
V.B.S S.75
S 50
WELL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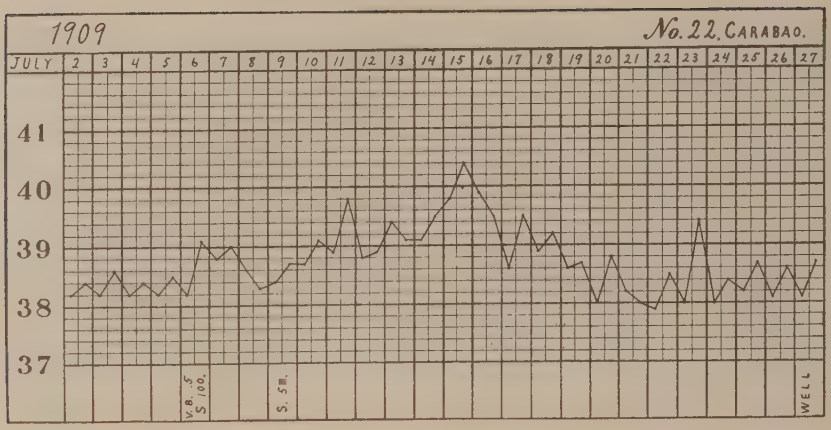
S. 50.
WELL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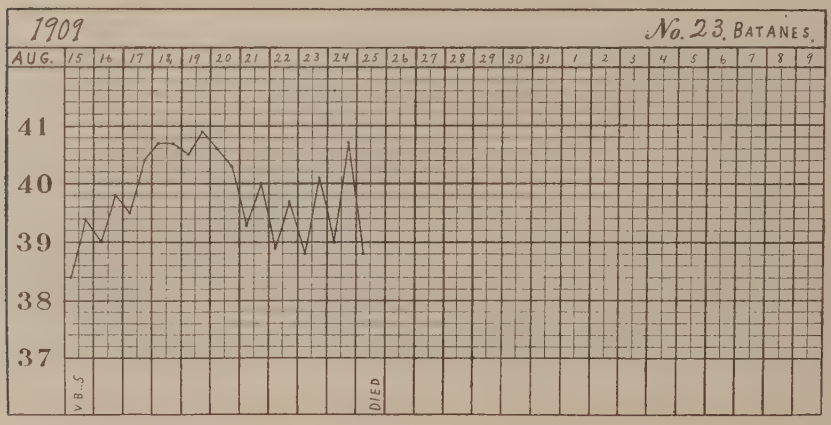
S. SH.
WELL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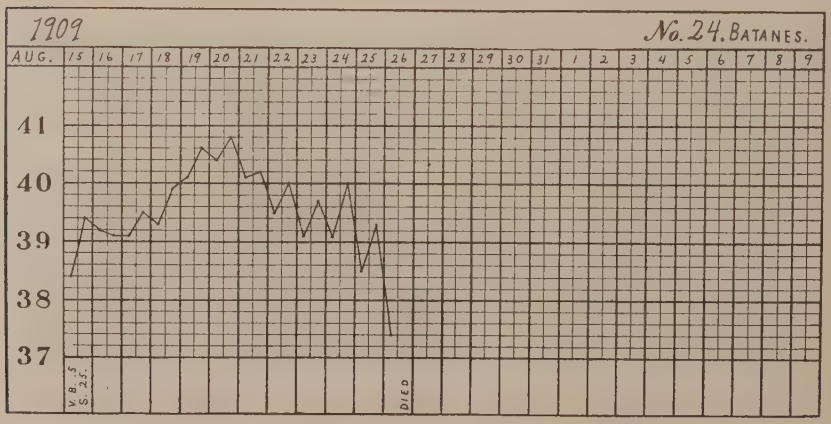
37
V.B..S
DIED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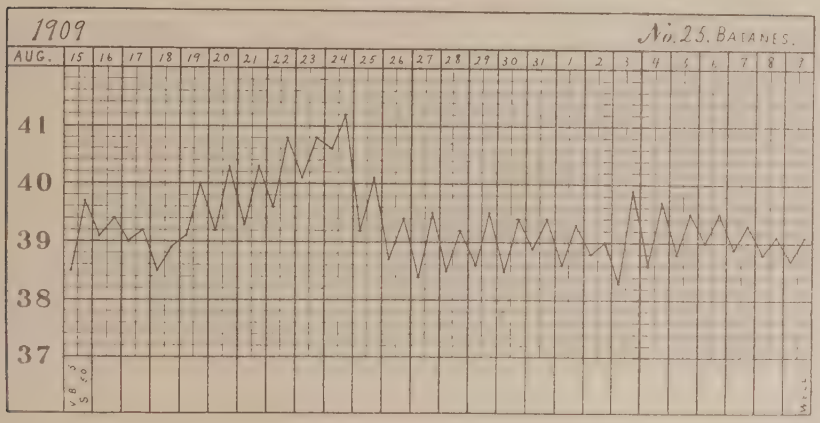
V.B..S S.225.
DIED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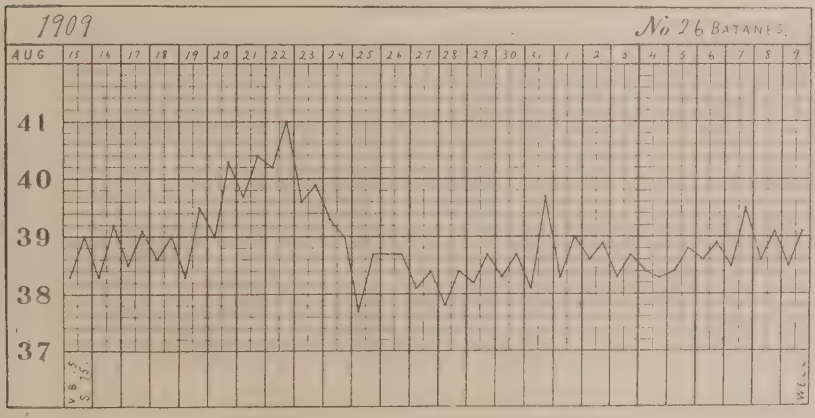
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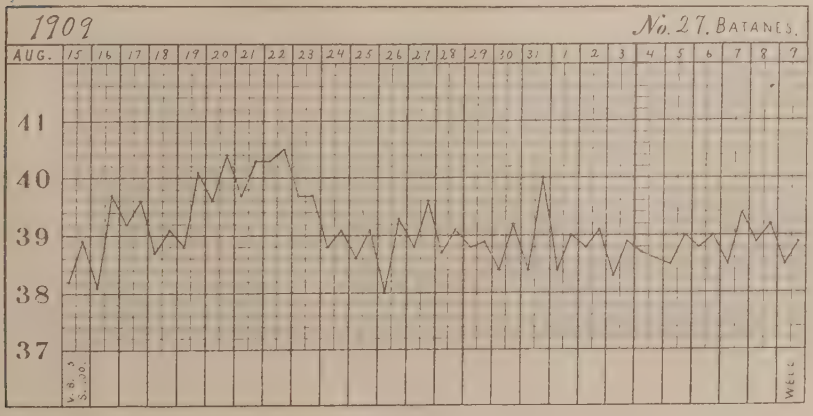
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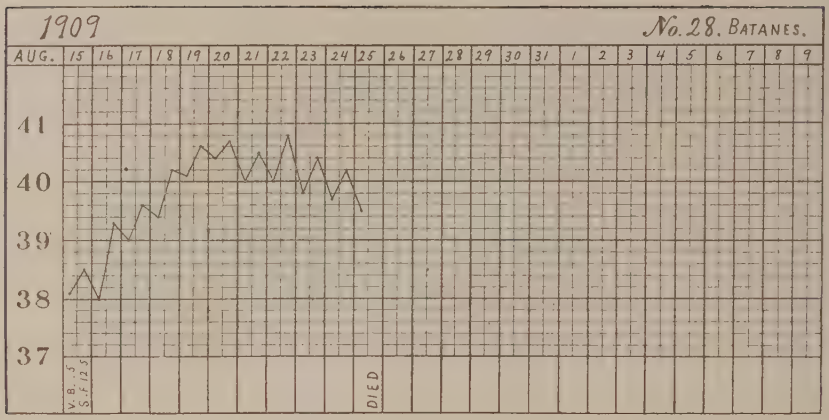
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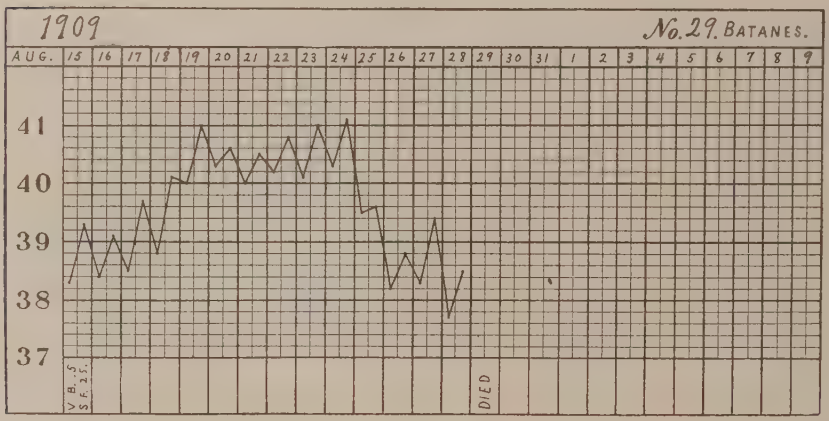
37
V. B. S
S. F. 12 S.
DIED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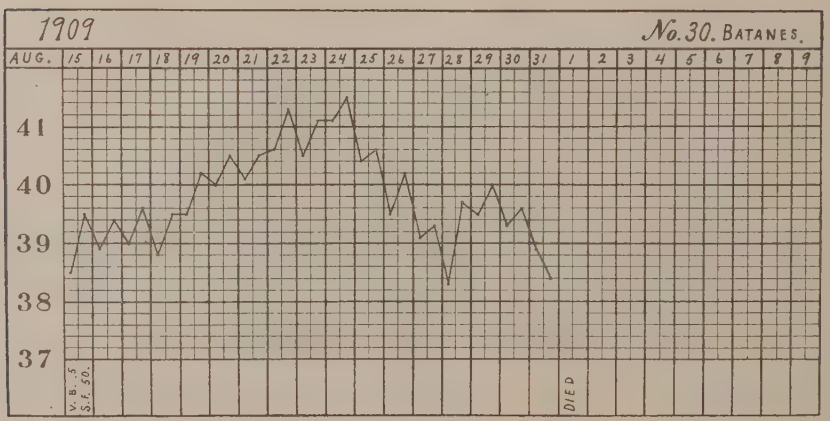
37
V. B. S
S. F. 25.
DIED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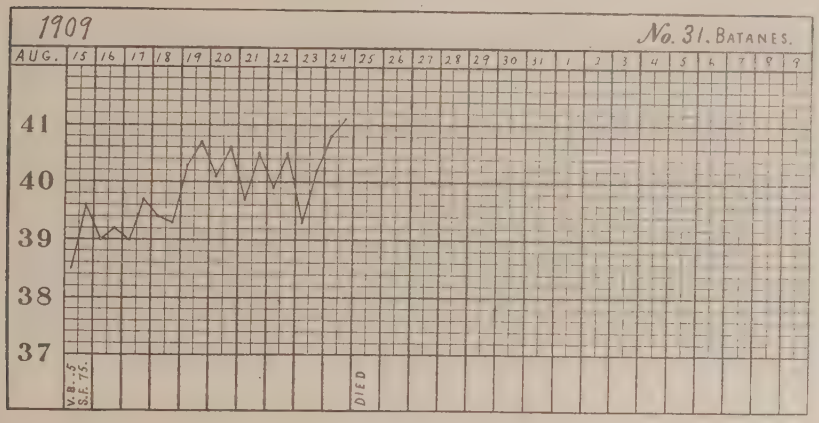
40
39
38
37
V. B. S
S. F. 50.
DIED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41
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39
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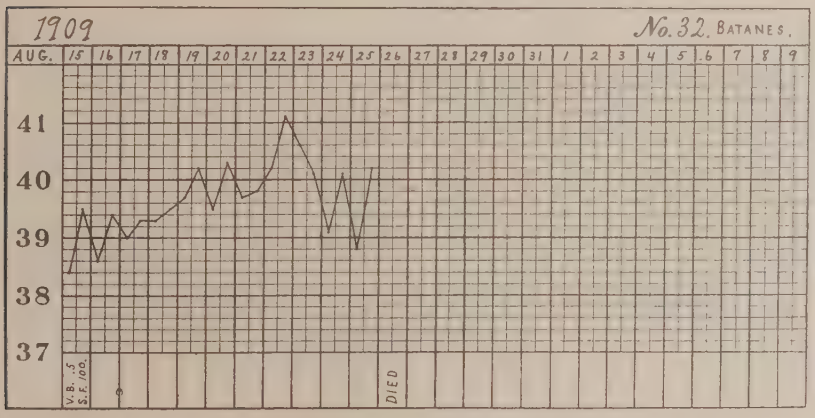
37
V.B. .5
S.F. 75.
DIED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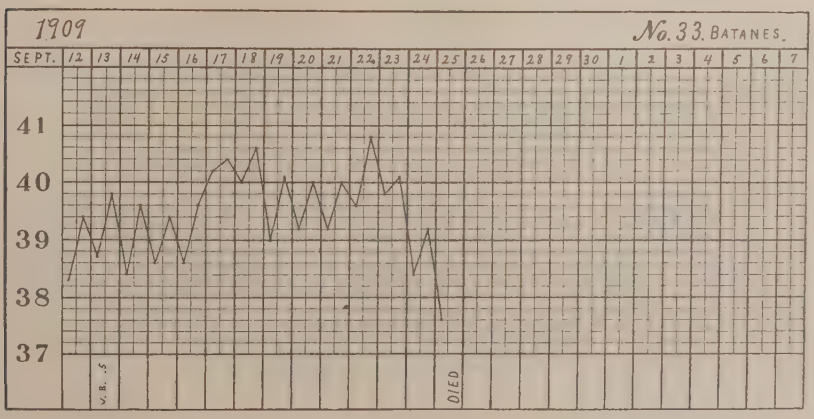
V.B. .5
S.F. 100.
DIED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41
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39
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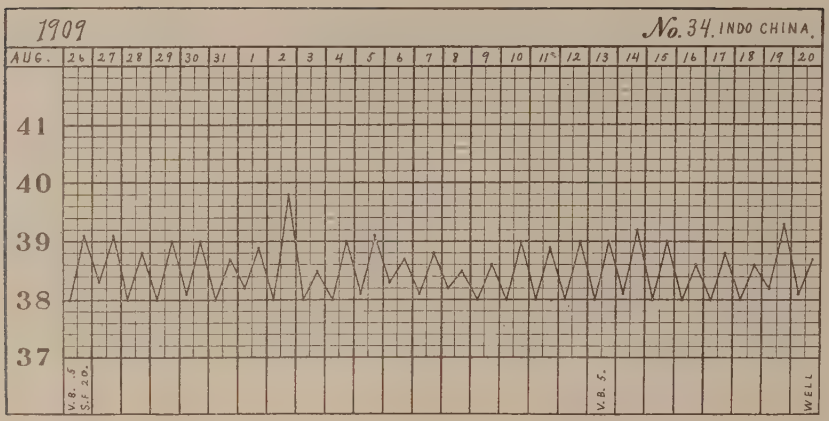
37
V.B. .5
DIED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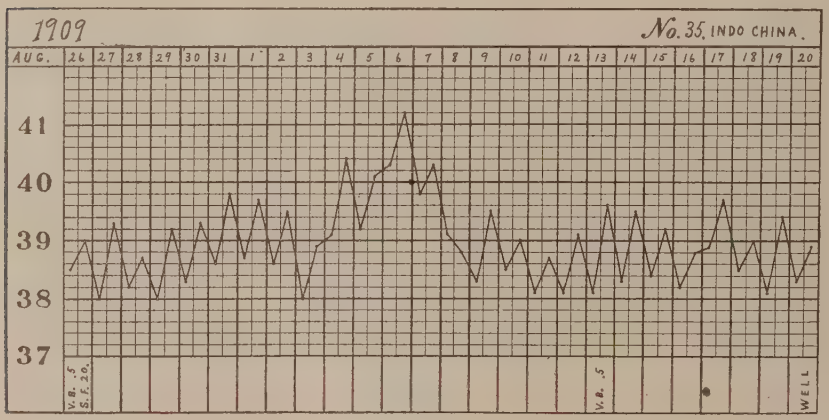
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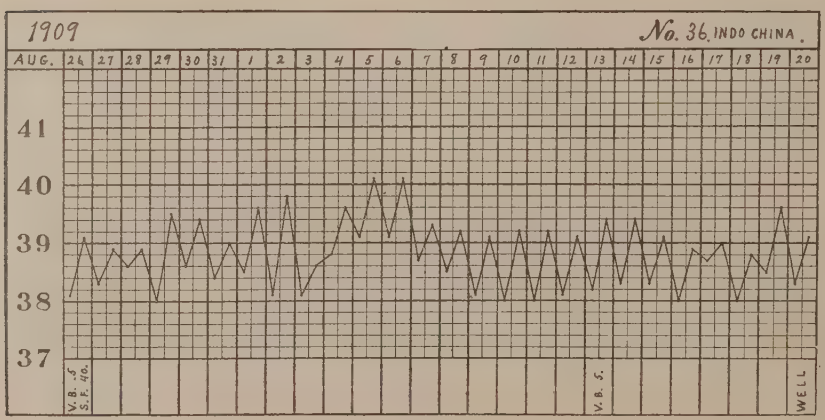
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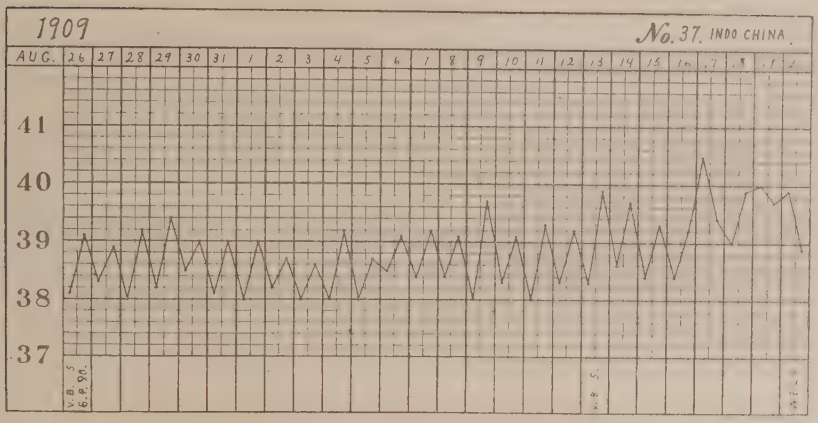
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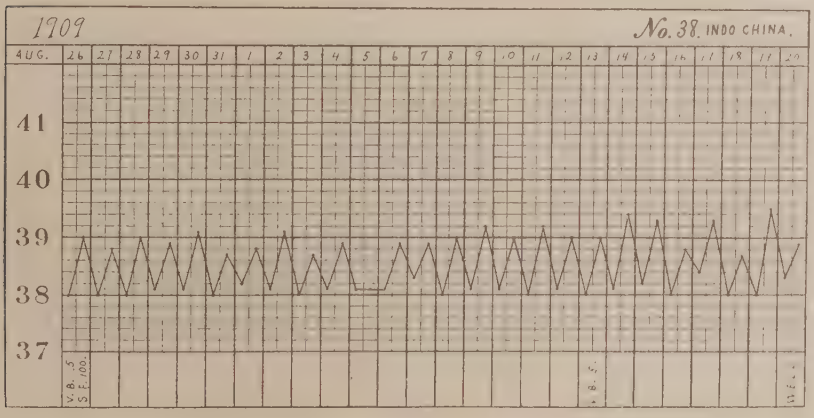
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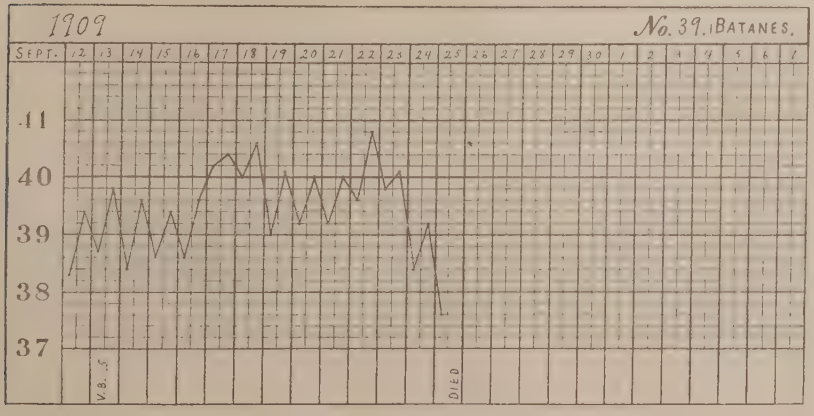
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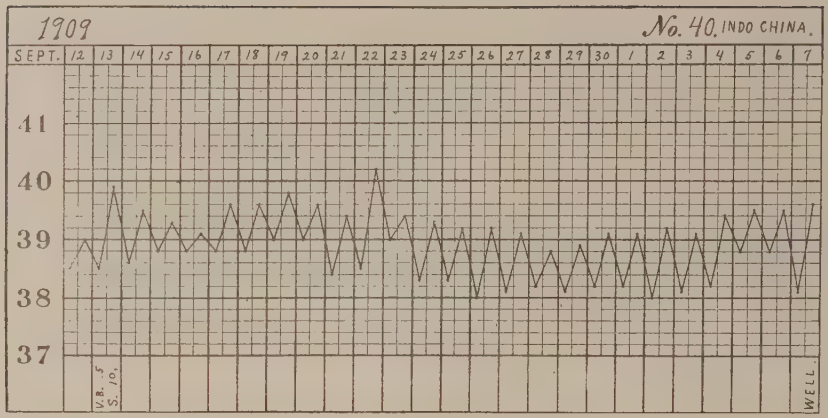
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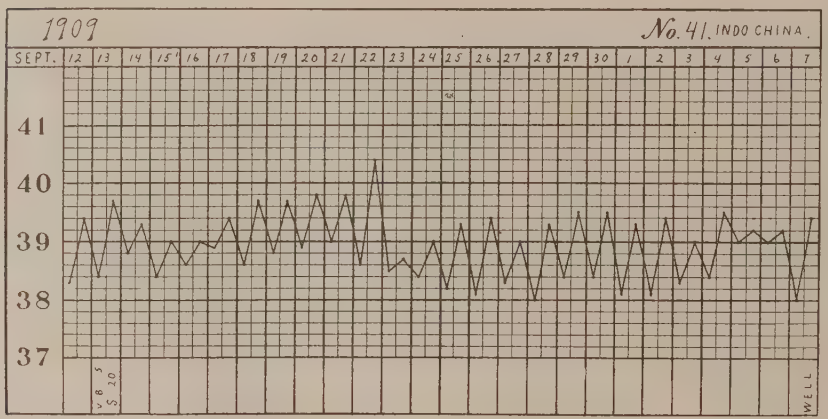
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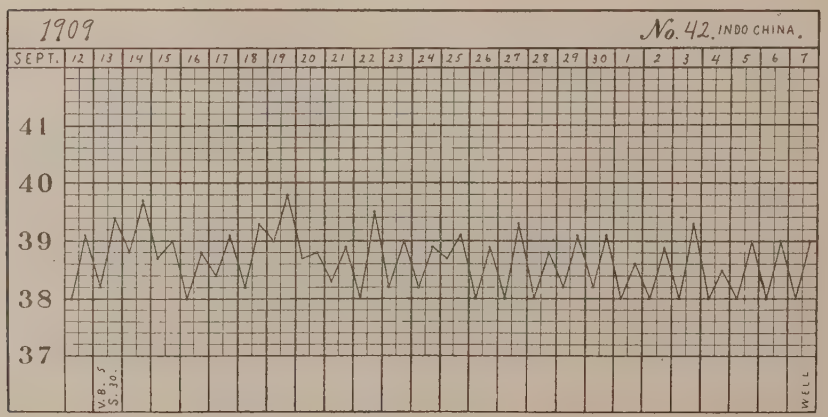
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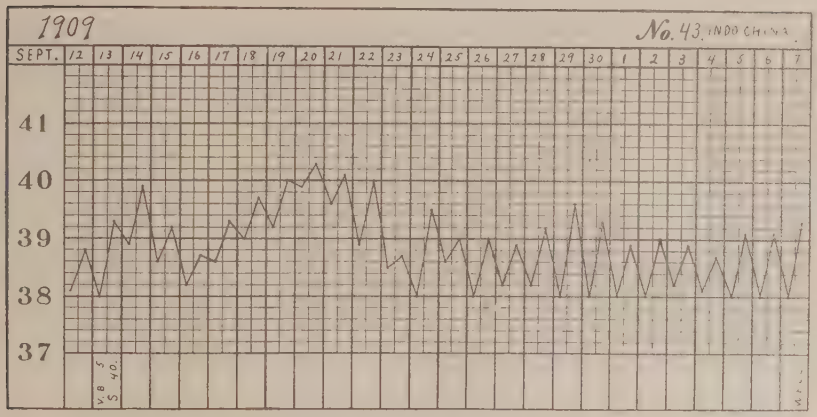
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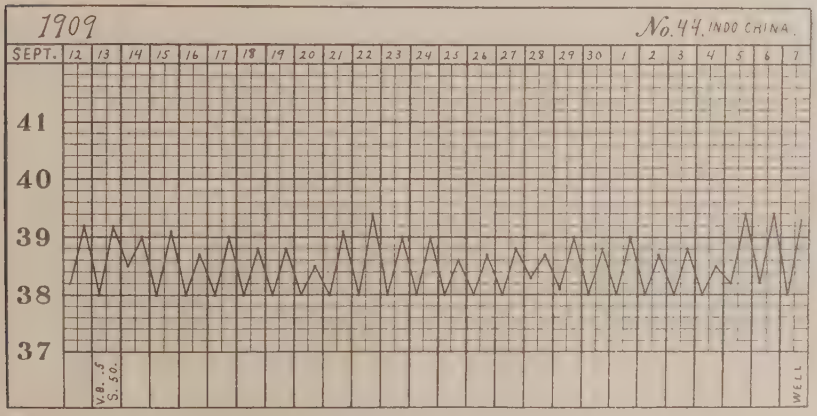
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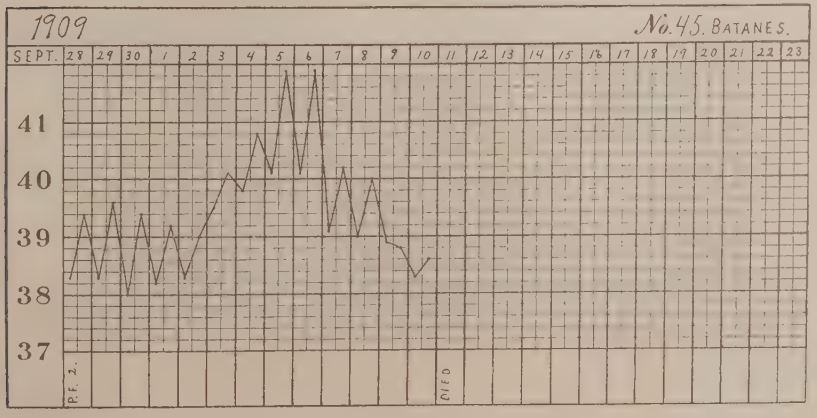
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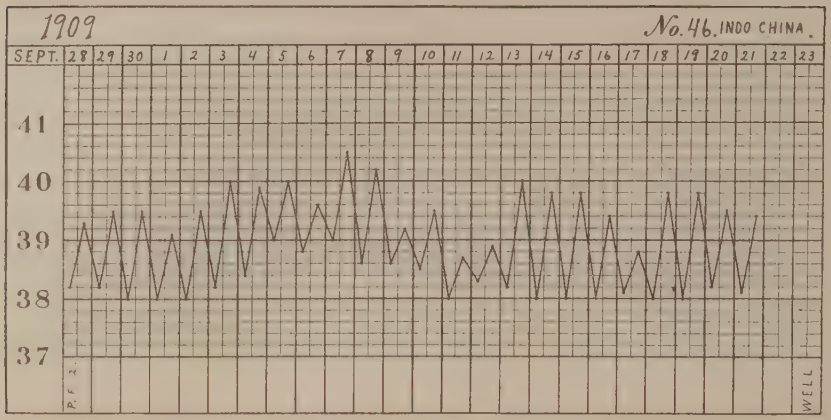
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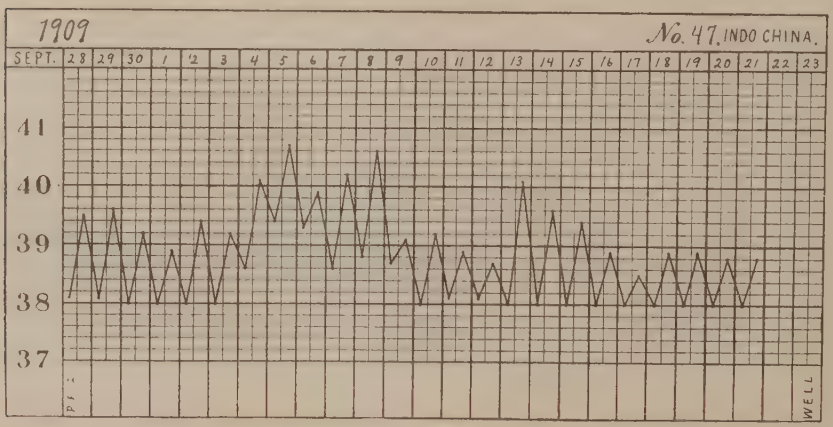
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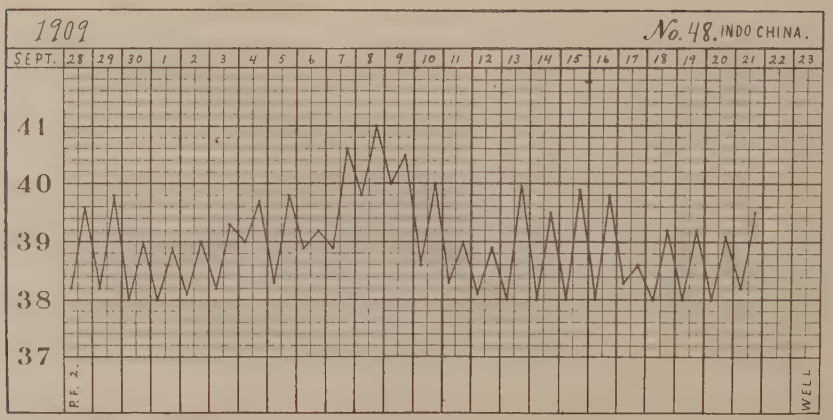
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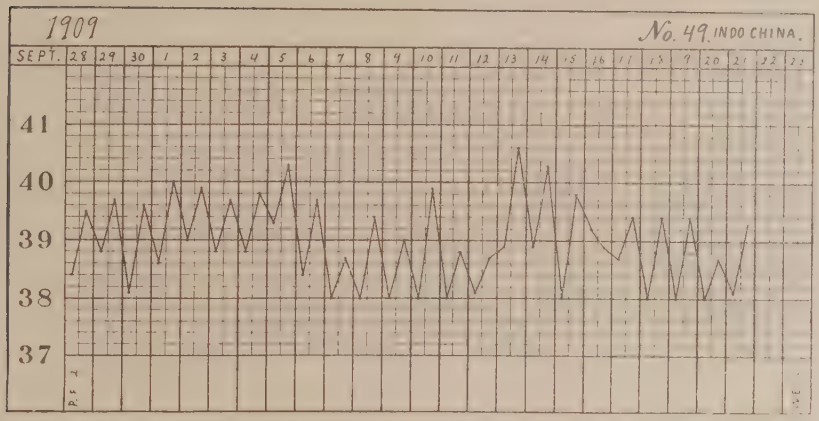
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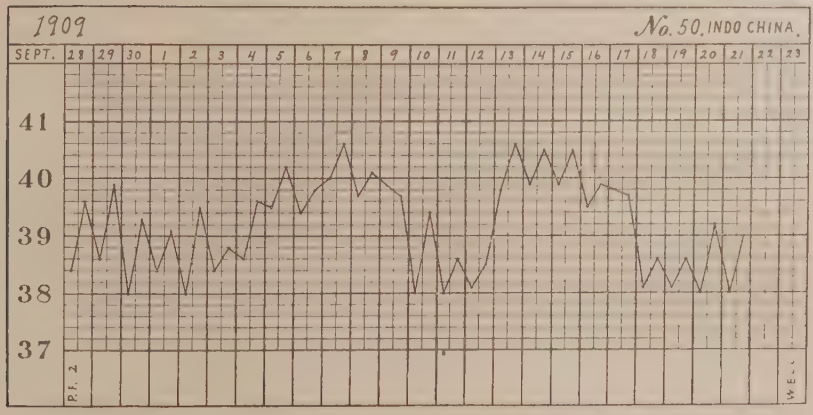
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TUBERCULOSIS IN THE PHILIPPINE ISLANDS.[1]

By EUGENE R. WHITMORE.[2]

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

Tuberculosis in its spread over the earth has not spared any nation or any climate, and the Philippine Islands have not been an exception to the rule. While cholera exists a large part of the time, and plague has occasionally appeared here, tuberculosis is always present, and causes far more suffering and many more deaths than any other disease.

The death rate from tuberculosis in Manila is 5 per 1,000. In the year 1906–7, one-sixth of all deaths in Manila were from tuberculosis, and one-seventh were from tuberculosis of the lungs. The following table compiled from the reports of the Director of Health shows the deaths from tuberculosis in Manila since 1903.

Period.	Population.[a]	Deaths.	Deaths from tuberculosis.	Deaths from pulmonary tuberculosis.
September 1, 1903, to August 31, 1904	219,941	10,781	1,153	958
September 1, 1904, to August 31, 1905	219,941	9,731	1,246	1,121
July 1, 1905, to June 30, 1906	219,941	9,486	1,355	1,209
July 1, 1906, to June 30, 1907	223,542	8,251	1,296	1,148
July 1, 1907, to June 30, 1908	223,542	9,351	1,222	1,076

[a] Population for 1901, 1905 and 1906 is from the official census of Manila for 1903. Population for 1907 and 1908 is from the health census for 1907.

It is not easy to get satisfactory statistics of the prevalence of tuberculosis in the Philippines outside of Manila, but since the conditions which lead to the high death rate from tuberculosis in Manila do not exist to the same extent in the provinces, it is quite possible that tuberculosis is not as prevalent in the provinces as it is in Manila; however, we know that it is a very common disease throughout the Islands.

[1] Read at the Meeting of the Manila Medical Society, August 9, 1909.

[2] Captain, Medical Corps, United States Army; detailed to Biological Laboratory, Bureau of Science, Manila, P. I.

An important factor in the prevalence of tuberculosis here is the insanitary condition under which the people live. The majority of houses are greatly overcrowded, and at night all doors and windows are closed and the house is made as tight as it is possible to make a native bamboo structure. No precautions are taken to prevent contagion and it is not uncommon for ten to fifteen people to live and sleep in a small house of one room; sometimes from one to five of these people have tuberculosis and are coughing and spitting about the house so that all the inmates are constantly exposed to infection. Underfeeding also predisposes to the disease by lowering the resistance of the individual, but it can probably be truly said of the Philippines that "without the house, tuberculosis would not exist."

In 100 autopsies at the Philippine Medical School tuberculous lesions were found in 35.

The Bureau of Health of Manila is taking energetic steps to prevent the spread of tuberculosis. Overcrowding is prohibited, light and ventilation have been provided for, antispitting ordinances have been passed, and an active educational campaign is being carried out through the public schools and the daily press. It is also proposed to open a free dispensary in the city of Manila for tuberculous cases, and to erect one or more "tent colonies" for the open-air treatment of tuberculosis.

I am informed by the chief veterinarian of the Bureau of Agriculture that tuberculosis is very rare among the cattle in the Philippines—so rare as to be almost unknown, and also that tuberculosis is unknown among the carabaos, pigs, and goats. Furthermore, the natives drink little milk and eat little butter, and what milk they do drink is mainly derived from carabaos and goats. Still, surgical tuberculosis in man is by no means uncommon. In 1,400 surgical cases in St. Paul's Hospital, Manila, there have been 30 cases of joint tuberculosis, 27 cases of bone tuberculosis, and 32 cases of cervical and axillary gland tuberculosis. Among 732 patients in the University Hospital of this city there have been 5 cases of bone tuberculosis and 18 of cervical and axillary gland tuberculosis.

In the dispensary at the University Hospital for the year July 1, 1908, to June 30, 1909, there were 6,650 cases, of which 1,882 were surgical. Of 84 cases of chronic bone and joint disease 30 were instances of tuberculosis of the bones and joints. Of 92 cases of adenitis, 63 showed tuberculous lesions of the cervical and axillary glands.

Summing up the available (reliable) statistics on surgical tuberculosis in Manila, we obtain 187 cases of bone, joint, and gland tuberculosis out of a total of 4,014 surgical cases; i. e., a percentage of 4.65. This is certainly not a lower percentage than is found in Europe and

America. It is not possible to get any statistics on the amount of surgical as compared to medical tuberculosis, as comparatively few of the cases of pulmonary tuberculosis here are admitted to the hospitals. It has been considered by many writers that bone, joint and gland tuberculosis are produced by an infection with the bovine bacillus; the extreme rarity of tuberculosis in cattle in the Philippine Islands, together with the frequency of these types of infection in man, certainly speak against such a view.

Tuberculosis of the testicle is rather common in Manila—10 cases having been admitted to St. Paul's and 2 to the University Hospital. Doctor Saleeby of the latter institute informs me that tuberculosis of the mesenteric glands is unusually common here.

The following table shows the number of patients suffering with pulmonary tuberculosis during each of the past four years in Bilibid Prison, where over 3,000 prisoners are constantly confined.

	July 1, 1905, to June 30, 1906.	July 1, 1906, to June 30, 1907.	July 1, 1907, to June 30, 1908.	July 1, 1908, to June 30, 1909.
Remaining from previous year	100	153	153	156
Admitted	188	91	124	192
Died	84	40	58	86
Discharged	39	49	62	137
Released	12	2	1	1
Remaining for next year	153	153	156	124

Hygiene and diet are at present the chief factors in the treatment of tuberculosis, and, while specific treatment should logically form the basis, it has as yet shown no results which would entitle it to this position.

It would probably fill a volume even to mention the substances that have been advised in the specific treatment of tuberculosis. A few that are probably forgotten by most of us to-day are tannin, lead acetate, garlic, hydrogen sulphide, carbon dioxide, corrosive sublimate, boric acid, borax, carbolic acid, fluorides, camphor, etc. It generally happens that the amount of space devoted to the discussion of any of the specifics is inversely proportional to the length of time which has elapsed since the specific action was suggested. In order to test, in a comparative way, some of the more recently suggested specifics in the treatment of tuberculosis, I selected one hundred adult males who were suffering from pulmonary tuberculosis and were confined in the tuberculosis wards of the hospital at Bilibid Prison.

None of our cases were in the first stage; 80 were in the second;

and 20 were in the third stage of the disease. None of the cases showed any signs of syphilis.

The tubercle bacillus was found in the sputum of each patient before he was subjected to treatment, and von Pirquet's skin test was made on each. During the progress of the work, every man's temperature was taken twice a day, all were weighed once a week, and the sputum was examined once a month.

With regard to diet, clothing, habits of living and sleeping rooms, the patients were all under practically identical conditions. Hygienic and dietetic treatment are carried out with all patients in the hospital, and in April, 1909, a roof ward was opened, where the patients have protection from the sun and rain but are exposed to unobstructed air circulation from all sides.

An attempt was made to determine whether or not the method of complement fixation offers a means of differentiating between infection with the bovine and with the human type of the tubercle bacillus.

Twenty-eight of the 100 cases of pulmonary tuberculosis, 2 cases of bone tuberculosis, and 3 strong, healthy men were tested in regard to this point. Tubercle bacilli were present in the sputum of all the cases of pulmonary tuberculosis, and all of the cases of tuberculosis gave a positive skin reaction. The skin test was negative on all of the normal men. The blood serum of each man was tested for complement fixation with both human and bovine tuberculins (old) in the usual way with the usual controls (old tuberculins were used, as filtrates were not at hand[3]).

In the preliminary tests it was found that our tuberculins in dilution of 1 to 14 never gave fixation of complement when mixed with inactivated normal human serum diluted 1 to 5, using a hæmolytic system of goat corpuscles (5 per cent emulsion), anti-goat-rabbit serum and fresh guinea pig complement diluted 1 to 10.

The hæmolytic amboceptor was of such strength that 1 cubic centimeter of a 1 to 1,200 dilution completely hæmolyzed 1 cubic centimeter of corpuscle emulsion in the presence of 0.1 cubic centimeter of fresh guinea pig complement. As I was working with human sera it was not practical to obtain large amounts of serum, so I used 0.5 cubic centimeter of the serum dilution as suggested by Simon.[4] The above dilutions were employed throughout the work, using two dissolving doses of the hæmolytic amboceptor.

[3] Détre's differential skin test is made with filtrates of bouillion cultures of human and bovine tubercle bacilli. (*Wien. klin. Wchnsch.* (1908), **21**, 173, 1410.)

[4] *Journ. Exper. Med.* (1908), **10**, 673.

The following table shows the results of these tests:

Serum. Number.	Human tuberculin.	Bovine tuberculin.	Condition of person from whom serum was taken.
1	—		Normal.
2	—	—	Do.
3	tr.	slt.	Normal. Physician working in tuberculosis wards.
4	+	+ + +	Tuberculosis of bones of ankle, sinus, recovering. Had recovered 6 months later.
5	tr.	slt.	Tuberculosis of bones of ankle, no sinus, stationary, condition unchanged one year later.
6	—	—	Pulmonary tuberculosis.
7	slt.	—	Do.
8	tr.	slt.	Do.
9	slt.		Do.
10	+	—	Do.
11	+	—	Do.
12	+	slt.	Do.
13	slt.		Do.
14	slt.	+	Do.
15	—	slt.	Do.
16	+	slt.	Do.
17	+ +	+ +	Do.
18	slt.	+	Do.
19	+	+	Do.
20			Do.
21	+	+	Do.
22	+		Do.
23	+		Do.
24	+ +		Do.
25	+ +	+	Do
26	+	+	Do.
27	+ +	+	Do.
28	+	+ + +	Do
29	+	+	Do.
30	+ + +	+ +	Do.
31	—	+	Do.
32			Do.
33		—	Do.

— = No blocking of hæmolysis.
tr = trace of blocking of hæmolysis.
slt = slight blocking of hæmolysis.
+ = partial blocking of hæmolysis.
++ = nearly complete blocking of hæmolysis.
+++ = complete blocking of hæmolysis.

Excepting Nos. 10, 11, 32 and 33, there is fair correspondence between the degree of fixation of each serum with the two tuberculins.

The skin test was always made with both tuberculins and the bovine variety usually gave the stronger reaction, while the human seemed to give as marked fixation of complement as did the bovine.

The tuberculins used in these experiments had been in stock in the laboratory for a long time and the human tuberculin yielded only a slight skin reaction, while the bovine gave a well-marked one. Since both tuberculins yielded equally strong fixation of complement with the tuberculous sera, we believe that the experiments indicate that the substances in the tuberculins which are concerned with the fixation of complement reaction are not identical with those calling forth the cutaneous reaction.

It is seen from the table that the two tuberculins yielded approximately the same degree of fixation of complement with the various tuberculous sera and therefore the method is not an aid in differentiating the two types of infections. This can only be done by the isolation of the infecting organism in pure culture followed by a careful study of its cultural characteristics and pathogenicity for animals.

The cases treated were divided into five series of 20 cases each. They were, as nearly as possible, so assigned that the men of one series were in approximately the same physical condition and the same stage of the disease as were the men in any other series.

The first of the five series was further divided into two groups (A and B), receiving tuberculins by mouth and hypodermically, respectively.

The second series of twenty men was given succinimide of mercury according to the method of Wright[5] of the United States Navy.

The third series was given atoxyl or the similar compounds, soamin and arsacetin, intramuscularly.

The fourth series was given cinnamate of mercury intramuscularly. The cinnamate of mercury was made at my request by Doctor Bacon of the chemical laboratory of this Bureau. I hoped in this single drug to combine whatever of value there might be in both the Landerer and Wright methods of treatment. Landerer's idea was that the cinnamic acid caused a leucocytosis and set up an aseptic inflammation about the tuberculous focus resulting in cicatrization of the latter. He used chiefly sodium cinnamate (hetol) and preferred intravenous injection, although he used intramuscular injections almost exclusively for children. He considered that the intramuscular injections were of far less value than the intravenous, and that they were useless in severe cases. Landerer used small doses—25 milligrams, or rarely 50 milligrams as the maximum dose—while certain French writers have reported good results from using much larger doses.

Series 1, 2, 3, and 4 received no other medicinal treatment except an occasional cathartic or sedative cough mixture.

[5] *New York Med. Jour.* (1908), **88**, 385, and *U. S. Naval Med. Bull.* Wash. (1908), **2**, 1.

Series 5 was a control and the men were given the usual hospital treatment according to the individual case.

No patient was discharged as recovered until his cough had stopped, signs of an active lesion had disappeared and the sputum had been found negative for tubercle bacilli at least twice.[6]

During six months' treatment of these cases there have been twenty-one recoveries and nineteen deaths. Five men have been released from prison, leaving 55 of the cases still in the hospital.

The following table shows the number of recoveries, deaths and patients still in the hospital:

Series.	Died.	Returned to duty.	Remaining in hospital.	Number released, and condition on release.
1. Tuberculin:				
A. By mouth	0	8	5	1 gained 6 pounds.
B. Hypodermically	1	8	6	1 lost 8 pounds.
Total for tuberculin series.	1	6	11	2.
2. Succinimide of mercury	7	3	10	
3. Atoxyl	5	2	10	1 gained 38 pounds in 35 days; 1 gained 1 pound; 1 lost 4 pounds.
4. Cinnamate of mercury	2	6	12	
5. Control cases	4	4	12	
Total	19	21	55	5

From this table it is seen that the series treated with tuberculin and with cinnamate of mercury give the highest recovery rate; 6 of 20 cases, or 30 per cent in each instance.

The succinimide of mercury gives the highest death rate, with atoxyl and the control series following. The succinimide of mercury gives a recovery rate of 3 in 20 cases which is less than the control series, 4 in 20.

[6] When tubercle bacilli could not be found in the sputum by the ordinary method of examination, I have made use of the ligroin and antiformin methods. I now prefer a combination of the two methods as being clean, accurate and rapid. The sputum is treated with ten to fifteen times its volume of a 20 per cent solution of antiformin, a strongly alkaline solution of sodium hypochlorite with a content of about 4.2 per cent free chlorine. After standing until the sputum becomes homogeneous and fluid, about 2 cubic centimeters of ligroin are added and the mixture is shaken until a thorough emulsion is formed. Then the mixture is heated to 60° F. on a water bath until the ligroin has separated in a layer on top of the mixture. Several loops of the material in the limiting layer immediately at the lower border of the layer of ligroin are spread upon a warmed slide and fixed and stained in the usual way. It is often possible to find tubercle bacilli readily in a sputum by this method when they can not be found by the ordinary method of preparing the smear.

The attempt was made to give the succinimide of mercury, as advised by Wright, in doses of one-fifth of a grain every other day for fifteen doses, then two-fifths of a grain every four days for fifteen doses, then three-fifths of a grain once a week; but we very soon found that our cases would not stand that much mercury. In this connection it is well to remember that one-fifth of a grain of succinimide of mercury contains the same amount of the metal as one-eighth of a grain of bichloride of mercury, so we were not giving very large doses. Still our patients could not stand that amount of succinimide.

Possibly the succinimide does not cause as much induration and soreness at the site of injection, but it does not seem to have any other advantage over bichloride of mercury.

Thirty-eight of our cases had fever above 100° at the time treatment was started. Fifteen of these fever cases have died, while only 4 have died of the 62 patients who had normal temperature or fever of less than 100° when treatment was started. Only one man who had fever above 100° when treatment was started has been discharged as recovered. Of the remaining cases who had fever above 100°, 3 have remained stationary in weight, 11 have lost and 10 have gained weight.

From this short series it would seem that tuberculin had given the best results with one death and six recoveries; cinnamate of mercury next with two deaths and six recoveries; the control series next with four deaths and four recoveries; atoxyl next with five deaths and two recoveries; succinimide of mercury last with seven deaths and three recoveries; or possibly atoxyl and succinimide of mercury should change places.

We can not draw conclusions from so few cases, but it is evident that none of the methods of treatment we used is of very great value in the treatment of tuberculosis among the natives of the Philippines.

At a later date we hope to be able to give a full report of the final result in this series of one hundred cases.

I take this opportunity of thanking Dr. W. A. Christensen, physician at Bilibid Prison, for valuable assistance rendered throughout the work.

NOTICE.

The president of the American Gynecological Society has appointed a committee to report at the next annual meeting in Washington, on the present status of obstetrical teaching in Europe and America, and to recommend improvements in the scope and character of the teaching of obstetrics in America.

The committee consists of the professors of obstetrics in Columbia University, University of Pennsylvania, Harvard, Jefferson Medical College, Johns Hopkins University, Cornell University and the University of Chicago.

Communications from anyone interested in the subject will be gladly received by the chairman of the committee, Dr. B. C. Hirst, 1821 Spruce street, Philadelphia, Pennsylvania.

REVIEW.

Practical Bacteriology, Blood work, and Animal Parasitology. By E. R. Stitt, A. B., Ph. G., M. D. Cloth, pages XI–294, 86 illustrations, $1.50 net. Philadelphia; P. Blakiston's Son & Co., 1909.

In this little volume of less than 300 pages Doctor Stitt has given clinical methods for laboratory diagnosis such as is required of the average medical man.

This book is especially adapted for use by physicians in the tropics, where there are few well-equipped clinical laboratories, where every man must do most of his own laboratory work and must improvise most of his apparatus and where comparatively few books are available for reference. Doctor Stitt's large experience in the tropics makes his writing from that standpoint of great value. The key at the beginning of each chapter on study and identification of bacteria is a great help in practical work, especially to the man who has not had the advantage of laboratory training.

The four parts on bacteriology, study of the blood, animal parasitology, and clinical bacteriology and animal parasitology of the various body fluids and organs, are accurate, concise and practical treatises on the various subjects named. Blank leaves for notes have been put in at the end of each part.

The illustrations are good and the printing is well done on good paper. The book contains a large amount of information that is not contained in text-books, or is scattered through a number of books. Very few errors have crept into the work and it can be heartily recommended as a practical laboratory guide.

E. R. W.

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[NOTE.—The heavy-faced type is used in this index only in cases where the species is new, that is, pertaining to this year.]

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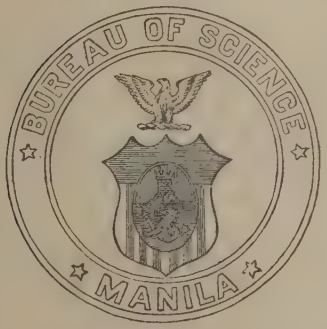

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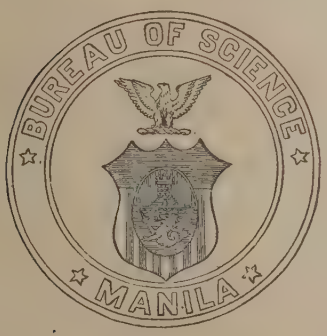

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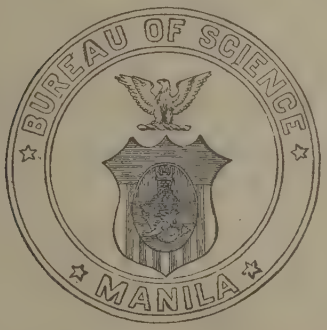

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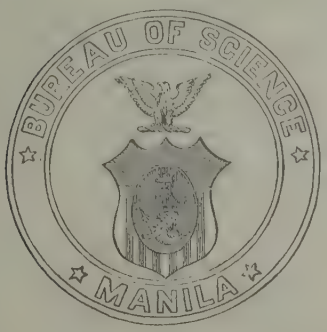

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